User Guide: Liquids NMR

Varian NMR Spectrometer Systems
With VNMR 6.1C Software

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Technical contributors: Greg Brissey, Steve Cheatham, Bayard Fetler, Phil Hornung, Dan Iverson, Boban John, Frits Vosman, Evan Williams
Technical writers: Michael Carlisle, Everett Schreiber
Technical editor: Dan Steele

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Overview of Contents

SAFETY PRECAUTIONS 25

Introduction 29

Chapter 1. Advanced 1D NMR 31

Chapter 2. 1D Experiments 73

Chapter 3. Multidimensional NMR 89

Chapter 4. Multidimensional and Advanced Experiments 137

Chapter 5. Indirect Detection Experiments 171

Chapter 6. Data Analysis 201

Chapter 7. Pulse Analysis 225

Chapter 8. Variable Temperature Operation 259

Chapter 9. Carousel, SMS, and NMS Automation 267

Chapter 10. VAST Accessory Operation 311

Chapter 11. PFG Modules Operation 371

Chapter 12. PFG Modules Experiments 393

Chapter 13. LC-NMR Accessory Operation 401

Chapter 14. LC-NMR Accessory Experiments 455

Index 477

Table of Contents

SAFETY PRECAUTIONS 25

Introduction 29

Chapter 1. **Advanced 1D NMR** 31

1.1 Working with Experiments 31

1.2 Multi-FID (Arrayed) Spectra 32

Arrayed Parameters 32

Multiple Arrays 33

Setting Array Order and Precedence 34

Interactively Arraying Parameters 34

Resetting an Array 34

Array Limitations 35

Acquiring Data 35

Processing 35

Display and Plotting 35

Saving and Retrieving 36

Pulse Width Calibration Step-by-Step 37

1.3 T_1 and T_2 Analysis 37

Setting Up The Experiment 38

Processing the Data 38

Analyzing the Data 38

Exponential Analysis Menu 39

T_1 Data Workup: Step-by-Step 39

1.4 Kinetics 39

Setting Up the Experiment 39

Processing the Data 40

Kinetics Step-by-Step 40

1.5 Diffusion Experiments 40

Pulsed Gradient Experiments 40

Pulsed Gradient Experiment Setup 42

Gradient Calibration 44

Data Reduction 44

Data Display 47

Variations on the pge Pulse Sequence 48

DOSY Experiments 48

Filter Diagonalization Method 68

Using FDM 68

Chapter 2. **1D Experiments** 73

2.1 APT—Attached Proton Test 74

Applicability 74

Parameters 74

Technique 74

References 74
Related Commands and Macros 75
2.2 BINOM—Binomial Water Suppression 75
Applicability 75
Parameters 75
Reference 75
2.3 CPMGT2—Carr-Purcell Meiboom-Gill T_2 Measurement 76
Applicability 76
Parameters 76
T_2 Measurement 76
Acquisition and Processing 76
2.4 CYCLENOE—Cycled NOE Difference Experiment 77
Applicability 77
Parameters 77
Technique 78
2.5 D2PUL—Standard Two-Pulse Using Decoupler as Transmitter 78
Applicability 78
Parameters 79
Technique 79
2.6 DEPT—Distortionless Enhancement by Polarization Transfer 79
Applicability 79
Parameters 80
Technique 80
Potential Problems 81
Reference 81
Related Commands and Macros 81
2.7 INEPT—Insensitive Nuclei Enhanced by Polarization Transfer 82
Applicability 82
Parameters 82
Technique 83
Reference 83
2.8 JUMPRET—Jump-and-Return Water Suppression 83
Applicability 83
Parameters 83
Reference 83
2.9 NOEDIF—NOE Difference Experiment 84
Applicability 84
Parameters 84
Phase Cycling 84
Procedure 85
Reference 87
2.10 PRESAT—1D Water Suppression 87
Applicability 87
Parameters 87
2.11 S2PUL—Standard Two-Pulse Sequence 87
Applicability 87
Parameters 87
2.12 S2PULR—Standard Two-Pulse in Reverse Configuration 88
Applicability 88

Parameters 88
Technique 88

Chapter 3. Multidimensional NMR 89

3.1 Interferograms 89
3.2 2D NMR Step-by-Step 90
To Process Stored Data 90
To Acquire a Simple COSY 91
3.3 Phase-Sensitive 2D NMR 91
3.4 Data Acquisition: Arrayed 2D 92
Hypercomplex Method 92
TPPI Method 93
Real-Time 2D 94
Macros for 2D Experiments 94
3.5 Weighting 95
Parameters 95
Setting Values 95
Interactive Weighting 96
3.6 Phasing Before the 2D Transform 97
3.7 Baseline Correction 98
First-Point Multiplier 99
Baseline Correction 100
FID Drift Correction 100
Spectral Drift Correction 101
3.8 Processing Phase-Sensitive 2D and 3D Data 101
Processing Programs 103
Common Coefficients for wft2d Processing 104
Sign of f_1 Frequencies 105
2D Solvent Subtraction Filtering 106
Left Shift, Frequency Shift, Phase Rotation 106
2D Processing of 3D Data 106
3.9 2D and 3D Linear Prediction 107
3.10 Phasing the 2D Spectrum 108
3.11 Display and Plotting 109
Display Modes 109
Display and Plot Limits 109
Maximum Intensity 111
Axis Label and Direction 111
Display Scaling 112
Grid Lines 112
Color Maps and Contour Plots 112
Whitewashed Spectra 113
Label Display 113
Projection of 2D Data 113
2D Referencing 114
Rotating Homonuclear 2D-J Spectra 114
Symmetrizing Data 114
Setting Negative Intensities to Zero 114
Automatic Analysis 115

3.12 Interactive 2D Color Map Display 115
Interactive 2D Display Menus 117
Controlling the Display with the Mouse 118
Changing the Display 118
Treating 2D Traces as 1D Spectra 120
3.13 Interactive 2D Peak Picking 120
Interactive 2D Peak Picking Menus 123
Automatic 2D Peak Picking 127
Interactive Peak Picking or Editing 127
Automatic Integration 128
Interactive Integration and Editing 128
Labeling and Commenting Peaks 128
Displaying Peaks in dconi 129
Peak File Manipulations 129
3.14 3D NMR 129
3D Acquisition 130
3D Processing 131
3D Display 132
3D Pulse Sequences 132
Experiment Setup 132
Data Processing 133
3.15 4D NMR Acquisition 134

Chapter 4. Multidimensional and Advanced Experiments 137

4.1 Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY 138
Applicability 139
Parameters 139
Technique 139
Potential Problems 141
4.2 COSY—Correlated Spectroscopy 141
Applicability 141
Parameters 141
Technique 141
4.3 COSYPS—Phase-Sensitive COSY 141
Applicability 141
Parameters 141
Phase Cycling 142
Technique 143
References 143
4.4 DQCOSY—Double-Quantum Filtered COSY 143
Applicability 143
Parameters 143
Phase Cycling 144
Technique 144
Potential Problems 145
References 145
4.5 HET2DJ—Heteronuclear 2D-J 145
Applicability 145
Parameters 145

Technique 146
Potential Problems 146
References 147
4.6 HETCOR—Heteronuclear Chemical Shift Correlation 147
Applicability 147
Parameters 147
Technique 148
Potential Problems 149
References 149
4.7 HETCORPS—Absolute-Value and Phase-Sensitive HETCOR 149
Applicability 150
Parameters 150
Recommendations 150
4.8 HOM2DJ—Homonuclear J-resolved 2D 150
Applicability 150
Parameters 151
Technique 151
Potential Problems 152
References 152
4.9 INADEQUATE—Double-Quantum Transfer Experiment 152
Applicability 152
Parameters 152
Technique 153
4.10 MQCOSY—Multiple-Quantum Filtered COSY 153
Applicability 153
Parameters 153
Technique 154
References 154
4.11 NOESY—Nuclear Overhauser Effect Spectroscopy 155
Applicability 155
Parameters 155
Phase Cycling 156
Technique 156
Potential Problems 157
Reference 158
4.12 ROESY—Rotating Frame Overhauser Effect Spectroscopy 158
Applicability 158
Parameters 158
Phase Cycling 159
Technique 159
Reference 160
4.13 TNCOSYPS—COSYPS with Water Suppression 160
Applicability 160
Parameters 160
4.14 TNDQCOSY—DQCOSY with Water Suppression 160
Applicability 160
Parameters 160
4.15 TNMQCOSY—MQCOSY with Water Suppression 161
Applicability 161

Parameters 161
References 161
4.16 TNNOESY—NOESY with Water Suppression 161
Applicability 162
Parameters 162
4.17 TNROESY—ROESY with Water Suppression 162
Applicability 162
Parameters 162
Technique 163
4.18 TNTOCSY—TOCSY with Water Suppression 163
Applicability 163
Parameters 163
References 164
4.19 TOCSY—Total Correlation Spectroscopy 164
Applicability 164
Parameters 164
Phase Cycling 165
Technique 165
References 165
Related Macros 165
4.20 TROESY—Transverse ROESY 166
Applicability 166
Parameters 166
Reference 166
4.21 HCCHTOCSY Pulse Sequence 166
Applicability 166
Parameters 166
Technique 167
4.22 HMQCTOCSY Pulse Sequence 168
Applicability 168
Parameters 168
Technique 168
4.23 HMQC-TOCSY 3D Pulse Sequence 168
Applicability 168
Parameters 168
Technique 169
4.24 HSQC-TOCSY 3D Pulse Sequence 169
Applicability 169
Parameters 170

Chapter 5. Indirect Detection Experiments 171

5.1 Requirements for Indirect Detection Experiments 171
Probes 172
RF System 172
Pulse Sequences 174
HMQC Pulse Sequence 174
HMQCR Pulse Sequence 176
5.2 The Basic HMQC Experiment 177
Spin-Echo Difference Experiment 177

BIRD Nulling 179
Transmitter Presaturation for High-Dynamic Range Signals 180
5.3 Phase-Sensitive Aspects of the Sequence 181
5.4 Cancellation Efficiency 181
5.5 Pros and Cons of Decoupling 182
5.6 Specifications Testing 183
5.7 Using the HMQC and HMQCR Sequences 184
5.8 Recabling Single-Broadband Systems 185
5.9 Recabling Dual-Broadband Systems 185
5.10 Filters for Indirect Detection 186
5.11 Tuning the Probe in the Reverse Mode 186
Tune the ^{1}H Channel 186
Tune the X Channel 186
5.12 Controlling Transmitter Power in the Reverse Mode 187
5.13 Indirect Detection Calibration 187
5.14 Typical Experimental Protocol for HMQC Experiments 194
5.15 Differences for ^{15}N Indirect Detection 199
5.16 HSQC Experiment 199
Applicability 199
Parameters 199

Chapter 6. Data Analysis 201

6.1 Spin Simulation 201
Spin Simulation Step-by-Step 202
Spin Simulation Menus 204
Entering a Spin System 204
Spin Simulation Parameters 204
Performing a Spin Simulation 205
Iterative Mode 205
Spin Simulation Files 206
6.2 Deconvolution 207
Deconvolution Step-by-Step 207
Performing Deconvolution 208
Display and Plotting 209
Deconvolution Menu 210
6.3 Reference Deconvolution 210
Reference Deconvolution of 1D Spectra 211
Reference Deconvolution of 2D Spectra 212
References 213
6.4 Addition and Subtraction of Data 213
Add/Subtract Menu 213
Noninteractive Add/Subtract 214
Interactive Add/Subtract 216
6.5 Regression Analysis 218
Regression Commands and Menus 218
Regression Analysis Step-by-Step 219
Contents of “analyze.out” File 221
Contents of “regression.inp” File 223

6.6 Chemical Shift Analysis 224

Chapter 7. Pulse Analysis 225

7.1 Pulse Shape Analysis 225
Directory and File Operations 226
Attribute Selection 227
Scale and Reference 227
Cursors 227
Simulation Overview 228
Simulation Parameters 228
Performing a Simulation 229
Creating a Pulse 230
7.2 Pandora's Box 230
Getting Started 231
Calibrating the RF Field 231
Creating Waveforms from Macros 232
Creating Waveforms from UNIX 233
Pbox File System 233
Pbox VNMR Parameters 237
Wave String Variables 239
Creating Waveforms Using Menus 240
Pbox Macro Reference 242
Pbox PSG Statements 243
Pulse Shaping "On-Fly" 254
Pbox_psg.h include Pulse Sequence Statements 254
shonfly.c Sequence 256
Pbox UNIX Commands 258

Chapter 8. Variable Temperature Operation 259

8.1 Startup 259
8.2 Operating Procedures 260
8.3 Temperature-Related Command 262
8.4 Operating Recommendations 263
8.5 VT Controller Safety Circuits 264
8.6 VT Interlock Parameters 265

Chapter 9. Carousel, SMS, and NMS Automation 267

9.1 Carousel Autosampler 267
Configuring VNMR for the Carousel 268
Checking Out the Carousel 269
Mounting and Removing the Carousel 271
Adjusting the Eject Air 272
Loading and Unloading Samples 273
Running NMR on One Sample at a Time 274
Running Automated NMR on Up to Nine Samples 275
Inserting Samples Manually with the Carousel Attached 276
Carousel Error Codes and Recovery 277
9.2 SMS Autosampler 279
Configuring VNMR for the SMS Autosampler 279
Preparing Sample Tubes 280

Running NMR on One Sample at a Time 280
Running Automated NMR 281
SMS Error Codes and Recovery 282
9.3 NMS Autosampler 283
Before Using NMS 284
Configuring VNMR for the NMS Autosampler 284
Running NMR on One Sample at a Time 285
Running Automated NMR 285
9.4 General Automation Tasks For All Sample Changers 287
Preparing and Initiating an Automation Run 287
Setting Up an Automation Run for Multiple Users 288
Monitoring an Automation Run 289
Using Sample Changers in Continuous Walkup Mode 291
Adding Samples to an Automation Run in Progress 292
9.5 Changing Sample Changers or Serial Ports 293
9.6 Using Gradient Autoshimming with Automation 293
9.7 Automation Run Description 294
Basic Automation Run 295
Automation Behind the Scenes 296
While an Automation Run is in Progress 296
When an Automation Run is Finished 297
Parameters for Automation 297
Variable Temperature Control During Automation 298
9.8 Customizing the Sample Entry Window 299
9.9 Automated Data Acquisition 299
Optimizing Acquisition Macros 301
Customizing Macro Operation 301
Example of Customizing a Macro 303
9.10 Automated Data Processing 306
9.11 File Structures in an Automation Run 307

Chapter 10. VAST Accessory Operation 311

10.1 Using the VAST Accessory 311
To Prepare VAST for Use 312
To Set Up NMR Experiments for VAST 314
To Change Samples with VNMR 314
To Shut Down a VAST System 316
10.2 Solvent Suppression in VAST 317
Setting Up Solvent Suppression 317
Troubleshooting Solvent Suppression 321
Evaluating Solvent Mixture Equilibration 322
Solvent Suppression: Background Information 322
10.3 Processing, Displaying, and Plotting VAST Data Sets 323
Creating a Pseudo 2D Data Set 323
Processing, Displaying, and Plotting Glued VAST Data 325
Defining a Custom Display Order with `plate_glue` 327
Examples of Plots of a VAST Data Set 328
Summary of VAST Display and Plot Options 329
10.4 Using CombiPlate to Analyze Data 331

Preparing VNMR Data For Analysis Using CombiPlate 331
Data Analysis Using CombiPlate And VNMR 332
Analyzing Data Using CombiPlate Without VNMR. 334
Checking And Fixing The Color Map. 336
10.5 Vast Process, Display, and Plot Macros 337
10.6 Preparing the Hardware and Configuring VNMR 341
Connecting the Transfer Tube 341
Connecting the Air Tubing 342
Connecting Signal and Power Cables 342
Configuring VNMR for VAST 344
10.7 Calibrating Volumes and Flow Rates 345
To Calibrate Probe Volume 345
To Calibrate Sample Volume 350
To Calibrate Flow Rate Parameters 351
To Calibrate XYZ Positions of the Arm 354
10.8 Acquiring Data on Standard Test Samples 354
10.9 Evaluating Carryover 355
10.10 VAST Interface Description 355
SAMPLE Def. 356
Rack Def. Pane 359
Main Control 359
Calibrations 361
10.11 Customizing the enter Window for VAST 362
10.12 Files that Control VAST Operation 363
10.13 Writing VAST Protocols 363

Chapter 11. PFG Modules Operation 371

11.1 Configuring the Software 371
11.2 PFG Amplifier Operation 372
11.3 Shimming PFG Systems 374
Performa I and Performa II 374
Performa XYZ 374
11.4 Setting Up Software for Imaging Pulse Sequences 374
Calibrating the Gradients 374
Creating a Gradient Table 374
Setting the System Gradient Coil 375
11.5 Homospoil Gradient Type 375
11.6 Gradient Shimming 376
Configuring Gradients and Hardware Control 377
Gradient Shimming Method 377
Mapping the Shims 377
Starting Gradient Shimming 378
Quitting the Gradient Shimming System Menu 378
General User Gradient Shimming 379
How Gradient Shimming Works 379
References 380
How Making a Shimmap Works 381
Shimmap Files and Parameters 382
How Automated Shimming Works 383

Deuterium Gradient Shimming 383
Homospoil Gradient Shimming for ^{1}H or ^{2}H 385
Full Deuterium Gradient Shimming Procedure for Lineshape 386
Setting Up Automation 386
Suggestions for Improving Results 387
Gradient Shimming Menus 388

Chapter 12. PFG Modules Experiments 393

12.1 GCOSY—PFG Absolute-Value COSY 393
Parameters 393
Processing 394
12.2 GHMQC—PFG HMQC 394
Parameters 394
Processing 394
12.3 GHMQCPS—PFG HMQC, Phase Sensitive 394
Processing 395
Recommendations 395
12.4 GHSQC—PFG HSQC, Absolute Value or Phase Sensitive 395
Parameters 395
Processing 396
12.5 GMQCOSY—PFG Absolute-Value MQF COSY 396
Parameters 396
Processing 396
12.6 GNOESY—PFG NOESY 397
Parameters 397
Processing 397
12.7 GTNNOESY—PFG TNNOESY 398
Parameters 398
Processing 398
12.8 GTNROESY—PFG Absolute-Value ROESY 398
Parameters 398
Processing 400
12.9 PFG Selective Excitation 400
Parameters 400
Reference 400

Chapter 13. LC-NMR Accessory Operation 401

13.1 LC-NMR Experiments Overview 402
13.2 Solvent Suppression in LC-NMR 404
Setting Up Solvent Suppression 404
Troubleshooting Solvent Suppression 408
Evaluating Solvent Mixture Equilibration 408
Solvent Suppression: Background Information 409
13.3 Maximizing LC Sensitivity 410
13.4 On-Flow LC-NMR Experiments 410
Acquiring Isocratic On-Flow LC-NMR Data 411
Acquiring Gradient On-Flow LC-NMR Data 413
13.5 Stop-Flow LC-NMR Experiments 414
Setting The Threshold and Slice Width Values 416

Acquiring Stop-Flow LC-NMR Data Using Scout 417
Acquiring Stop-Flow LC-NMR Data Using Enter 418
13.6 Time-Slice Stop-Flow Experiments 421
Creating a Time-Slice Program in Cascade 421
Acquiring Time-Slice Stop-Flow Data—Completely Automatic 421
Acquiring Time-Slice Stop-Flow Data—Semiautomatic 422
Acquiring Time-Slice Stop-Flow Data—Manually 425
13.7 2D LC-NMR Experiments 425
Acquiring 2D LC-NMR Data Manually 426
Acquiring 2D LC-NMR Data Using enter Program 426
13.8 Analyte Collector Operation 427
Initializing the Analyte Collector 427
Performing Analyte Collection 427
Performing Analyte Analysis 428
13.9 Data Processing Methods 429
Isocratic Data 430
Solvent Gradient (Scout Scan) Data and Stopped Flow Data 430
Manually Processing Gradient On-Flow (Scout-Scan) Data 430
Processing Data from an Aborted LC Experiment 430
NMRgram 430
Displaying or Plotting LC-NMR Data 431
13.10 Delay Time Calibration 431
Calibrating from the LC Detector to the Microflow Probe 431
Calibrating from the LC Detector to the Analyte Collector 433
Calibration from the Analyte Collector to the Microflow Probe 434
New Solvent Concentration 435
13.11 Chromatography for LC-NMR 435
Standards Used for LC-NMR Chromatography 435
Starting the LC pump 436
Setting Up STAR 5.31 436
Printing a Chromatogram 440
Typical Chromatograms 441
Setting Up an Automation Run in STAR Chromatography 441
Hints for Good LC-NMR Chromatography 442
13.12 Cascade LC-NMR Stop-Flow Program 443
Using the Cascade Program 443
Cascade LC-NMR Monitor Window 445
System Configuration 447
Time Program 450
Batch Scheduler 450
Communications 451
Analyte Collector Window 451
Initializing the Analyte Collector 452
Analyte Collection 452
Analyte Analysis 453

Chapter 14. LC-NMR Accessory Experiments 455

14.1 LC1D—LC-NMR 1D Pulse Sequence 455
Applicability 455

Macro 455
Parameters 455
Calibrations 456
14.2 WET1D—Water Eliminated through Transverse Gradients 1D 457
Applicability 457
Macro 457
Parameters 457
14.3 WETDQCOSY—WET Double-Quantum Filtered COSY 458
Applicability 458
Macro 458
Parameters 458
Phase Cycling 459
Technique 459
Potential Problems 460
References 460
14.4 WETGCOS—WET PFG Absolute-Value COSY 460
Applicability 460
Macro 460
Parameters 461
Processing 462
14.5 WETGHMQCPS—WET PFG HMQC (Phase Sensitive) 462
Applicability 462
Macro 462
Parameters 462
Processing 463
14.6 WETGHSQC—WET PFG HSQC, Absolute Value or Phase Sensitive 463
Applicability 463
Macro 463
Parameters 463
Processing 464
14.7 WETGMQCOSY—WET PFG Absolute-Value MQF COSY 465
Applicability 465
Macro 465
Parameters 465
Processing 466
14.8 WETNOESY—WET Nuclear Overhauser Effect Spectroscopy 466
Applicability 466
Macro 466
Parameters 466
Phase Cycling 467
Technique 468
Potential Problems 469
Reference 469
14.9 WETPWXAL—WET Pulse Width X Channel Calibration 469
Applicability 469
Macro 469
Parameters 470
Technique 470
14.10 WETRELAYH—WET Relay-COSY, WET Double Relay-COSY 471

Applicability 471
Macro 471
Parameters 471
Technique 472
Potential Problems 474
14.11 WETTNTOCS—WET TOCSY with Water Suppression 474
Applicability 474
Macro 474
Parameters 474
References 475

Index 477

List of Figures

Figure 1. PGE Pulse Sequences 41
Figure 2. Data Processing Macros Flowchart 45
Figure 3. Sample `analyze.inp` File 45
Figure 4. Gradient Stimulated Echo Element 49
Figure 5. Tcl-Tk Acquisition Panel of Doneshot Pulse Sequence 52
Figure 6. Dbppste Experiment 52
Figure 7. DgcsteSL Experiment 53
Figure 8. Oneshot DOSY Experiment 54
Figure 9. Tcl-Tk Process Panel for 2D_DOSY Pulse Sequences 57
Figure 10. Tcl-Tk Acquisition Panel for the Dgcstecosy Pulse Sequence 59
Figure 11. Dgcstecosy (AV Mode) Experiment 59
Figure 12. Dgcstehmqc Experiment (AV Mode) 60
Figure 13. Tcl-Tk Process2 Panel for the 3D-DOSY Pulse Sequences 62
Figure 14. `fdm1.inparm` File 71
Figure 15. APT Pulse Sequence 74
Figure 16. BINOM Pulse Sequence 75
Figure 17. CPMGT2 Pulse Sequence 76
Figure 18. D2PUL Pulse Sequence 78
Figure 19. DEPT Pulse Sequence 79
Figure 20. INEPT Pulse Sequence 82
Figure 21. NOEDIF Pulse Sequence 84
Figure 22. S2PULR Pulse Sequence 88
Figure 23. Data Flow in Phase-Sensitive 2D Transformation 101
Figure 24. Interactive 2D Contour Display (dconi Program) 116
Figure 25. Interactive 2D Peak Picking (ll2d Program) 123
Figure 26. Absolute-Value COSY Pulse Sequence 138
Figure 27. RELAY- COSY Pulse Sequences 138
Figure 28. COSYPS Pulse Sequence 142
Figure 29. DQCOSY Pulse Sequence 143
Figure 30. HET2DJ Pulse Sequence 145
Figure 31. HETCOR Pulse Sequence 147
Figure 32. HOM2DJ Pulse Sequence 151
Figure 33. INADEQUATE Pulse Sequence 152
Figure 34. MQCOSY Pulse Sequence 154
Figure 35. NOESY Pulse Sequence 155
Figure 36. ROESY Pulse Sequence 158
Figure 37. TOCSY Pulse Sequence 164
Figure 38. HMQC-TOCSY 3D Pulse Sequence 168
Figure 39. HMQC Pulse Sequence with `null`<>0 and `mbond='n'` 175
Figure 40. HMQCR Pulse Sequence 177

Figure 41. Heteronuclear Spin-Echo Difference Experiment 178
Figure 42. HMQC Pulse Sequence, Showing Movement of Attached Protons 178
Figure 43. Evolution Time Added Between X-Nucleus Pulses 179
Figure 44. HMQC with BIRD Pulse Nulling Effects 180
Figure 45. Basic HMQC Pulse Sequence 184
Figure 46. Normal ^{13}C Spectrum of $^{13}CH_3I$ 188
Figure 47. Normal ^{1}H Spectrum of $^{13}CH_3I$ 188
Figure 48. HMQC Without and With X-Nucleus Pulses 189
Figure 49. Calibration of `pwx`, Coarse and Fine 190
Figure 50. HMQC 4-Pulse Cancellation without ^{2}H Bandpass Filter 191
Figure 51. HMQC 4-Pulse Cancellation with ^{2}H Bandpass Filter 191
Figure 52. HMQC Without and With ^{13}C Decoupling 192
Figure 53. Coupled HMQC Spectrum of $^{13}CH_3I$ 193
Figure 54. Verifying Cancellation with `pwx=0,90` 195
Figure 55. Optimizing the BIRD Nulling Time 196
Figure 56. Coupled HMQC Spectrum of 3-Heptanone 197
Figure 57. Expansion of Coupled 3-Heptanone HMQC Showing Multiplets 198
Figure 58. Decoupled HMQC Spectrum of 3-Heptanone 198
Figure 59. Interactive Add/Subtract Display (addi Program) 216
Figure 60. Display of Regression Fittings (expl Program) 220
Figure 61. Temperature Control Window 263
Figure 62. Carousel Autosampler Carousel and Driver 268
Figure 63. Carousel with Sensor Arm in Locked and in Run Positions 269
Figure 64. Lock Pin on Sensor Arm Disengaged and Engaged 269
Figure 65. Bottom View of Carousel, Showing Connector and Groove 270
Figure 66. Optical Sensor and Proper Sample Floating Height 272
Figure 67. Sample Entry Form Window for the Carousel Autosampler 275
Figure 68. Manually Loading and Unloading Samples Through Position 1 277
Figure 69. SMS Autosampler 279
Figure 70. Sample Entry Form Window (100 samples) for the SMS Autosampler 281
Figure 71. NMS Window 284
Figure 72. Sample Entry Form Window for the NMS Autosampler 286
Figure 73. Sample Status Window (`status` Program) 290
Figure 74. Locate Window (`status` Program) 291
Figure 75. Sample Entry Form Window for Walkup Operation 291
Figure 76. Gilson 215 Liquid Handler 312
Figure 77. VAST Sample Entry Form Window (enter program) 315
Figure 78. LC-NMR Pane for VAST 316
Figure 79. VAST Sequence Pane 318
Figure 80. VAST Pane 318
Figure 81. VAST Acq & Obs Pane 319
Figure 82. VAST Sequence Pane and Frequency Suppression Options 320
Figure 83. Partial `doneQ` File 324
Figure 84. Queue Name and Scout Directory Fields 324
Figure 85. `plate_glue` Window and `e4x4` glue order. 327
Figure 86. Array of 1D Spectra from a VAST Data Set 328

Figure 87. Plot of a Subset from a 96-Well Sample Plate 328
Figure 88. Contour Plot of a VAST Data Set 329
Figure 89. Stacked Plot of a VAST Data Set 330
Figure 90. Spectrum of the Sample in Well F6 330
Figure 91. CombiPlate Started by combishow From vnmr 332
Figure 92. CombiPlate Display - Binary Display For Each Region 333
Figure 93. CombiPlate Save File Window 334
Figure 94. CombiPlate Column and Row Input Screen 335
Figure 95. CombiPlate Field Box 335
Figure 96. CombiPlate Window for VAST Data Analysis 336
Figure 97. Connection Between the Rheodyne Injector Valve and Transfer Tube 341
Figure 98. VAST Air Connections 343
Figure 99. VAST Signal Cable Connections 344
Figure 100. Microflow Probe and Transfer Tube Volumes 345
Figure 101. Injecting Solution into a Microflow Probe 346
Figure 102. Microflow Probe Flow Cell 348
Figure 103. Finding the Optimum Sample Volume 352
Figure 104. Sample Definition Window, Expanded View 356
Figure 105. Rack Definition Window 360
Figure 106. Main Control Window 360
Figure 107. Calibrations Window 361
Figure 108. Gradient Shimming Pulse Sequence 379
Figure 109. Mapping the z1 Shim 380
Figure 110. Shimmap Plot 381
Figure 111. Curve Fit Plot 384
Figure 112. Display of Shim Adjustments for Each Iteration 384
Figure 113. LC-NMR Block Diagram 402
Figure 114. lc1d Sequence Pane 405
Figure 115. LC-NMR Pane 405
Figure 116. lc1d Acq & Obs Pane 406
Figure 117. lc1d Sequence Pane and Frequency Suppression Options 406
Figure 118. LC-NMR Sample Entry Form Window 419
Figure 119. Process LC-NMR Button in LCNMR/STARS Pane 429
Figure 120. LC-NMR Spare Pane 431
Figure 121. Profile Showing Delay Time is Correct 432
Figure 122. Profile Showing the Delay Time is Too Short 433
Figure 123. Profile Showing the Delay Time is Too Long 433
Figure 124. STAR System Control Window 438
Figure 125. STAR Configuration Window 438
Figure 126. STAR Pump Method Window 439
Figure 127. Detector Method Windows 439
Figure 128. Isocratic Run of the Three Benzoates with the 9050 UV-Vis Detector 441
Figure 129. STAR Sequence List Window 442
Figure 130. Cascade Monitor Window 446
Figure 131. Cascade System Configuration Window 448
Figure 132. Time Program Window 450

Figure 133. Batch Scheduler Window 451
Figure 134. Communications Window 451
Figure 135. Analyte Collector Window 452
Figure 136. LC1D Pulse Sequence 456
Figure 137. WETDQCOSY Pulse Sequence 458
Figure 138. WETGCOSY Pulse Sequence 461
Figure 139. NOESY Pulse Sequence 466
Figure 140. Absolute-Value COSY Pulse Sequence 471
Figure 141. RELAY- COSY Pulse Sequences 471

List of Tables

Table 1. Commands and Macros for Working with Experiments 31
Table 2. Arrayed Spectra Commands and Parameters 33
Table 3. T_1 and T_2 Analysis Commands and Parameters 37
Table 4. Kinetics Analysis Commands and Parameters 40
Table 5. Diffusion Analysis Commands 41
Table 6. Gradient Control Parameter Set 42
Table 7. Tools for the DOSY Experiment 49
Table 8. Dbppste Parameters 53
Table 9. DgcsteSL Parameters 53
Table 10. Oneshot DOSY Parameters 54
Table 13. Dgcstecosy Parameters 59
Table 14. Dgcstehmqc Parameters 60
Table 16. Arrayed 2D & 3D Data Acquisition Commands and Parameters 93
Table 17. Macros for 2D Experiments 94
Table 18. Weighting Parameters for ni and ni2 Dimensions 95
Table 19. Commands and Macros for Setting 2D Weighting Values 96
Table 20. Commands and Parameters for Phasing Before the 2D Transform 98
Table 21. Baseline and Drift Correction Commands and Parameters 99
Table 22. Tools for Processing Phase-Sensitive 2D and 3D Data 102
Table 23. 2D and 3D Linear Prediction (LP) Commands and Parameters 107
Table 24. Commands and Parameters for Phasing the 2D Spectrum 108
Table 25. 2D Display and Plotting Commands and Parameters (Part 1 of 2) 110
Table 26. 2D Display and Plotting Commands and Parameters (Part 2 of 2) 111
Table 27. Interactive 2D Color Map Display Commands and Parameters 116
Table 28. Interactive 2D Peak Picking Commands and Parameters 120
Table 29. 3D NMR Commands and Parameters (Part 1 of 2) 130
Table 30. 3D NMR Commands and Parameters (Part 2 of 2) 131
Table 31. 4D NMR Acquisition Commands and Parameters 135
Table 32. Parameters for HMQC and HMQCR Pulse Sequences 184
Table 33. System Bandpass Filters for Indirect Detection Probes. 186
Table 34. Parameter Values for HMQCR on Natural Abundance Sample 197
Table 35. Spin Simulation Commands and Parameters 202
Table 36. Deconvolution Commands and Parameters 209
Table 37. Reference Deconvolution Commands 211
Table 38. Add/Subtract Experiment Commands and Parameters 214
Table 39. Regression Commands 219
Table 40. Curve Types. 221
Table 41. Chemical Shift Analysis Commands 224
Table 42. Pulse Shape Analysis Commands and Parameters 225
Table 43. Pbox Commands and Parameters 258

Table 44. Variable Temperature Unit Operation Commands and Parameters 259
Table 45. Automation Mode Commands and Parameters 294
Table 46. Basic Parameters for Automation 298
Table 47. Commands and Parameters for Customizing Macro Operation 302
Table 48. Typical Default Values in Liquid Handler Windows 313
Table 49. Approximate Probe Volume Values for the Various Probes and Transfer Tubes 349
Table 50. Typical Flow Rates for Common Solvents 353
Table 51. Pulsed Field Gradients Commands and Parameters 372
Table 52. Homospoil Control 376
Table 53. Gradient Shim Availability 377
Table 54. Gradient Shimming Commands and Parameters 378
Table 55. Deuterium Parameters 385
Table 56. GCOSY Parameters 393
Table 57. GHMQC Parameters. 394
Table 58. GHSQC Parameters 396
Table 59. GMQCOSY Parameters 397
Table 60. GNOESY Parameters 397
Table 61. GTNNOESY Parameters 398
Table 62. GTNROESY Parameters 399
Table 63. Selective Excitation Parameters 400

SAFETY PRECAUTIONS

The following warning and caution notices illustrate the style used in Varian manuals for safety precaution notices and explain when each type is used:

WARNING: ***Warnings*** **are used when failure to observe instructions or precautions could result in injury or death to humans or animals, or significant property damage.**

CAUTION: ***Cautions*** **are used when failure to observe instructions could result in serious damage to equipment or loss of data.**

Warning Notices

Observe the following precautions during installation, operation, maintenance, and repair of the instrument. Failure to comply with these warnings, or with specific warnings elsewhere in Varian manuals, violates safety standards of design, manufacturing, and intended use of the instrument. Varian assumes no liability for customer failure to comply with these precautions.

WARNING: **Persons with implanted or attached medical devices such as pacemakers and prosthetic parts must remain outside the 5-gauss perimeter from the centerline of the magnet.**

The superconducting magnet system generates strong magnetic fields that can affect operation of some cardiac pacemakers or harm implanted or attached devices such as prosthetic parts and metal blood vessel clips and clamps.

Pacemaker wearers should consult the user manual provided by the pacemaker manufacturer or contact the pacemaker manufacturer to determine the effect on a specific pacemaker. Pacemaker wearers should also always notify their physician and discuss the health risks of being in proximity to magnetic fields. Wearers of metal prosthetics and implants should contact their physician to determine if a danger exists.

Refer to the manuals supplied with the magnet for the size of a typical 5-gauss stray field. This gauss level should be checked after the magnet is installed.

WARNING: **Keep metal objects outside the 10-gauss perimeter from the centerline of the magnet.**

The strong magnetic field surrounding the magnet attracts objects containing steel, iron, or other ferromagnetic materials, which includes most ordinary tools, electronic equipment, compressed gas cylinders, steel chairs, and steel carts. Unless restrained, such objects can suddenly fly towards the magnet, causing possible personal injury and extensive damage to the probe, dewar, and superconducting solenoid. The greater the mass of the object, the more the magnet attracts the object.

Only nonferromagnetic materials—plastics, aluminum, wood, nonmagnetic stainless steel, etc.—should be used in the area around the magnet. If an object is stuck to the magnet surface and cannot easily be removed by hand, contact Varian service for assistance.

Warning Notices (*continued*)

Refer to the manuals supplied with the magnet for the size of a typical 10-gauss stray field. This gauss level should be checked after the magnet is installed.

WARNING: **Only qualified maintenance personnel shall remove equipment covers or make internal adjustments.**

Dangerous high voltages that can kill or injure exist inside the instrument. Before working inside a cabinet, turn off the main system power switch located on the back of the console, then disconnect the ac power cord.

WARNING: **Do not substitute parts or modify the instrument.**

Any unauthorized modification could injure personnel or damage equipment and potentially terminate the warranty agreements and/or service contract. Written authorization approved by a Varian, Inc. product manager is required to implement any changes to the hardware of a Varian NMR spectrometer. Maintain safety features by referring system service to a Varian service office.

WARNING: **Do not operate in the presence of flammable gases or fumes.**

Operation with flammable gases or fumes present creates the risk of injury or death from toxic fumes, explosion, or fire.

WARNING: **Leave area immediately in the event of a magnet quench.**

If the magnet dewar should quench (sudden appearance of gasses from the top of the dewar), leave the area immediately. Sudden release of helium or nitrogen gases can rapidly displace oxygen in an enclosed space creating a possibility of asphyxiation. Do not return until the oxygen level returns to normal.

WARNING: **Avoid liquid helium or nitrogen contact with any part of the body.**

In contact with the body, liquid helium and nitrogen can cause an injury similar to a burn. Never place your head over the helium and nitrogen exit tubes on top of the magnet. If liquid helium or nitrogen contacts the body, seek immediate medical attention, especially if the skin is blistered or the eyes are affected.

WARNING: **Do not look down the upper barrel.**

Unless the probe is removed from the magnet, never look down the upper barrel. You could be injured by the sample tube as it ejects pneumatically from the probe.

WARNING: **Do not exceed the boiling or freezing point of a sample during variable temperature experiments.**

A sample tube subjected to a change in temperature can build up excessive pressure, which can break the sample tube glass and cause injury by flying glass and toxic materials. To avoid this hazard, establish the freezing and boiling point of a sample before doing a variable temperature experiment.

Warning Notices (*continued*)

WARNING: **Support the magnet and prevent it from tipping over.**

The magnet dewar has a high center of gravity and could tip over in an earthquake or after being struck by a large object, injuring personnel and causing sudden, dangerous release of nitrogen and helium gasses from the dewar. Therefore, the magnet must be supported by at least one of two methods: with ropes suspended from the ceiling or with the antivibration legs bolted to the floor. Refer to the *Installation Planning Manual* for details.

WARNING: **Do not remove the relief valves on the vent tubes.**

The relief valves prevent air from entering the nitrogen and helium vent tubes. Air that enters the magnet contains moisture that can freeze, causing blockage of the vent tubes and possibly extensive damage to the magnet. It could also cause a sudden dangerous release of nitrogen and helium gases from the dewar. Except when transferring nitrogen or helium, be certain that the relief valves are secured on the vent tubes.

WARNING: **On magnets with removable quench tubes, keep the tubes in place except during helium servicing.**

On Varian 200- and 300-MHz 54-mm magnets only, the dewar includes removable helium vent tubes. If the magnet dewar should quench (sudden appearance of gases from the top of the dewar) and the vent tubes are not in place, the helium gas would be partially vented sideways, possibly injuring the skin and eyes of personnel beside the magnet. During helium servicing, when the tubes must be removed, carefully follow the instructions and safety precautions given in the manual supplied with the magnet.

Caution Notices

Observe the following precautions during installation, operation, maintenance, and repair of the instrument. Failure to comply with these cautions, or with specific cautions elsewhere in Varian manuals, violates safety standards of design, manufacturing, and intended use of the instrument. Varian assumes no liability for customer failure to comply with these precautions.

CAUTION: **Keep magnetic media, ATM and credit cards, and watches outside the 5-gauss perimeter from the centerline of the magnet.**

The strong magnetic field surrounding a superconducting magnet can erase magnetic media such as floppy disks and tapes. The field can also damage the strip of magnetic media found on credit cards, automatic teller machine (ATM) cards, and similar plastic cards. Many wrist and pocket watches are also susceptible to damage from intense magnetism.

Refer to the manuals supplied with the magnet for the size of a typical 5-gauss stray field. This gauss level should be checked after the magnet is installed.

Caution Notices (*continued*)

CAUTION: **Keep the PCs, (including the LC STAR workstation) beyond the 5-gauss perimeter of the magnet.**

Avoid equipment damage or data loss by keeping PCs (including the LC workstation PC) well away from the magnet. Generally, keep the PC beyond the 5-gauss perimeter of the magnet. Refer to the *Installation Planning Guide* for magnet field plots.

CAUTION: **Check helium and nitrogen gas flowmeters daily.**

Record the readings to establish the operating level. The readings will vary somewhat because of changes in barometric pressure from weather fronts. If the readings for either gas should change abruptly, contact qualified maintenance personnel. Failure to correct the cause of abnormal readings could result in extensive equipment damage.

CAUTION: **Never operate solids high-power amplifiers with liquids probes.**

On systems with solids high-power amplifiers, never operate the amplifiers with a liquids probe. The high power available from these amplifiers will destroy liquids probes. Use the appropriate high-power probe with the high-power amplifier.

CAUTION: **Take electrostatic discharge (ESD) precautions to avoid damage to sensitive electronic components.**

Wear a grounded antistatic wristband or equivalent before touching any parts inside the doors and covers of the spectrometer system. Also, take ESD precautions when working near the exposed cable connectors on the back of the console.

Radio-Frequency Emission Regulations

The covers on the instrument form a barrier to radio-frequency (rf) energy. Removing any of the covers or modifying the instrument may lead to increased susceptibility to rf interference within the instrument and may increase the rf energy transmitted by the instrument in violation of regulations covering rf emissions. It is the operator's responsibility to maintain the instrument in a condition that does not violate rf emission requirements.

Introduction

This manual is designed to help you perform liquids NMR experiments on your Varian NMR spectrometer system using VNMR software (VNMR is Varian's NMR application software package). The manual contains the following chapters:

- Chapter 1, "Advanced 1D NMR," covers working with 1D experiments at a more advanced level, such as handling multi-FID spectra, kinetics, and T_1 and T_2 analysis.
- Chapter 2, "1D Experiments," describes a number of common 1D pulse sequences for "everyday" use.
- Chapter 3, "Multidimensional NMR," describes data acquisition, processing, and display for 2D and 3D NMR. Acquisition of 4D NMR is also covered.
- Chapter 4, "Multidimensional and Advanced Experiments," describes a number of 2D, 3D, and advanced liquids experiments.
- Chapter 5, "Indirect Detection Experiments," covers indirect detection experiments, also known as *heteronuclear multiple-quantum coherence* (HMQC) experiments.
- Chapter 6, "Data Analysis," covers spin simulation, deconvolution, addition and subtraction of data, regression analysis, and pulse shape analysis.
- Chapter 7, "Pulse Analysis," describes the Pulsetool and Pandora's Box tools.
- Chapter 8, "Variable Temperature Operation," describes VT startup and operation.
- Chapter 9, "Carousel, SMS, and NMS Automation," covers automation run acquisition and operation, including user programming of the sample entry window.
- Chapter 10, "VAST Accessory Operation," covers setting up and using the VAST sample changer.
- Chapter 11, "PFG Modules Operation," describes operation using Varian Pulsed Field Gradient modules.
- Chapter 12, "PFG Modules Experiments," describes experiments using PFG modules.
- Chapter 13, "LC-NMR Accessory Operation," describes operation using the LC-NMR accessory.
- Chapter 14, "LC-NMR Accessory Experiments," describes experiments that can be performed on a system equipped with the LC-NMR accessory.

Notational Conventions

The following notational conventions are used throughout all VNMR manuals:

- `Typewriter-like characters` identify VNMR and UNIX commands, parameters, directories, and file names in the text of the manual. For example:
  The `shutdown` command is in the `/etc` directory.
- `Typewriter-like characters` also show text displayed on the screen, including the text echoed on the screen as you enter commands. For example:
  `Self test completed successfully.`

- Text shown between angled brackets(<>) in a syntax entry is optional. For example, if the syntax is `seqgen s2pul<.c>`, entering the "`.c`" suffix is optional, and typing `seqgen s2pul.c` or `seqgen s2pul` is functionally the same.
- Lines of text containing command syntax, examples of statements, source code, and similar material might not fit the width of the page. In such cases, lines are broken at a convenient point (such as at a comma), a backslash (\) is inserted at the break, and the line is continued as the next line of text. This notation will be familiar to C programmers. The backslash is not part of the line and, except for C source code, should not be typed when entering the line.
- Because pressing the Return key is required at the end of almost every command or line of text you type, the Return key is mentioned only in cases where it is *not* used. This convention avoids repetition of the instruction "press the Return key."
- Text with a change bar (like this paragraph) identifies material new to VNMR 6.1C that was not in the previous version of VNMR. Refer to the *Release Notes* for a description of new features of the software.

Other Manuals

This manual should be your basic source of information on intermediate and advanced liquids NMR. Other VNMR 6.1 manuals you might need to reference include:

- *Getting Started*
- *VNMR Command and Parameter Reference*
- *Walkup NMR*
- *VNMR User Programming*
- *VNMR and Solaris Software Installation*
- *User Guide: Solid-State NMR*
- *User Guide: Imaging*

All of these manuals are shipped with the VNMR software. Note that these manuals, other Varian hardware and installation manuals, and most Varian accessory manuals are also provided online so that you can view the pages on your workstation and print copies.

Types of Varian Spectrometer Systems

In parts of this manual, the type of system (UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, *GEMINI 2000*, UNITY*plus*, UNITY, or VXR-S) must be considered in order to properly use the software.

- UNITY*INOVA* and *MERCURY-VX* are the current systems sold by Varian.
- UNITY*plus*, UNITY, and VXR-S are spectrometer lines that preceded the UNITY*INOVA*.
- *GEMINI 2000* is a separate line of spectrometers that preceded the *MERCURY* and *MERCURY-VX*.

Chapter 1. Advanced 1D NMR

Sections in this chapter:

- 1.1 “Working with Experiments,” this page
- 1.2 “Multi-FID (Arrayed) Spectra” on page 32
- 1.3 “T1 and T2 Analysis” on page 37
- 1.4 “Kinetics” on page 39
- 1.5 “Diffusion Experiments” on page 40

This chapter describes working with 1D NMR liquids experiments at a more advanced level than the manual *Getting Started*

1.1 Working with Experiments

Table 1 lists commands for working with experiments.

Table 1. Commands and Macros for Working with Experiments

Command	
`explib`	Display experiment library
`jexp1, jexp2, ...,jexp9999`	Join existing experiment
`md(<from_exp,>to_exp)`	Move display parameters between experiments
`mf(<from_exp,>to_exp)`	Move FIDs between experiments
`mp(<from_exp,>to_exp)`	Move parameters between experiments
Macros	
`cexp(<exp_dir,>exp_number)`	Create a VNMR experiment
`delexp(exp_number)`	Delete an experiment

To obtain a view of the experiments on a system, type `explib`. The monitor displays the experiment library of the currently available experiment files (`exp1`, `exp2`, ..., `exp9999`).

For each experiment, the following is displayed:

- The name of the experiment and its current size.
- The pulse sequence currently active in the experiment.
- The first 50 characters of the text file in the experiment.
- The names contained in subexperiments.

An experiment is created with the macro `cexp(n)`, where `n` is a number from 1 to 9999 (e.g., `cexp(4)` creates the experiment `exp4`). An experiment is deleted by the macro `delexp(n)`, where `n` is a number from 2 to 9999 (`exp1` cannot be deleted). For example, entering `delexp(4)` deletes `exp4`.

When multiple experiments are created, an issue arises concerning how to individually work with each experiment. To handle this matter, only one experiment is allowed at a time to be currently active (i.e., in the foreground for manipulation), although background processing can be occurring in other experiments at the same time.

To make another experiment currently active (provided it has been created), you "join" it by entering the `jexp` command with the experiment number as an argument. For example, to join `exp6`, enter `jexp6`.

The macros `jexp1` to `jexp9999` have a number of convenient uses. For example, they clear the graphics window, display a parameter set appropriate to the newly joined experiment, and restart the main menu. To prevent any of these actions, use the `jexp` command with an experiment number as an argument. For example, perhaps you want to simultaneously display a second spectrum from another experiment, but do not want the currently displayed spectrum to be erased. In this case, enter `jexp(6)` rather than `jexp6`.

The `mp`, `mf`, and `md` commands move FIDs and parameters between experiments:

- `mp(<n,>m)` moves parameters from experiment `n` to experiment `m`, for example, `mp(4,5)`. If `n` is omitted, parameters are moved from the currently active experiment to experiment `m`.
- `mf(<n,>m)` moves the last acquired FID and the associated parameters.
- `md(<n,>m)` moves only those "saved display" parameters associated with the commands `s1` through `s9`.

The Workspace menu contains most of the commands for working with experiments:

To enter this menu, click on the Main menu button, then select the Workspace button. You can also display this menu by entering the command `menu('workspace')`. The number of buttons in this menu depends on how many experiments have been created on your system. In the Workspace menu shown above, you see that five experiments have been created (remember that `exp1` is always present by default and cannot be deleted).

1.2 Multi-FID (Arrayed) Spectra

Many experiments require obtaining a series of FIDs, related to each other through the variation of one or more parameters. For example, suppose it is necessary to run a series of spectra at four different temperatures: 30°C, 50°C, 70°C, and 90°C. Instead of acquiring four separate sets of data, it is possible to create an array in which the `temp` parameter is given four successively different values. These four subexperiments are now all treated as a single experiment. Entering `go` begins successive acquisition of all four experiments. One command can be used to transform all the spectra, one command to display all the spectra on the screen simultaneously, one command to plot all the spectra, and one command to save all the spectra.

Arrayed Parameters

Table 2 lists command and parameters used with arrayed parameters.

To create an array for a numeric parameter, enter the arrayed values separated by commas, (e.g., `temp=30,50,70,90` or `pw=5,10,15,20,25`). Alphanumeric parameters can

Table 2. Arrayed Spectra Commands and Parameters

Commands	
`array*`	Easy entry of linearly spaced array values
`da<(param1<,param2,...>)>`	Display acquisition parameter arrays
`ds<(index)>`	Display trace from arrayed 1D spectra
`dss*`	Display stacked spectra
`dssa*`	Display stacked spectra automatically
`dssan*`	Display stacked spectra automatically without erasing
`dssh*`	Display stacked spectra horizontally
`dsshn*`	Display stacked spectra horizontally without erasing
`dssn*`	Display stacked spectra without erasing screen
`ft*`	Fourier transform 1D data
`pl*`	Plot spectra
`rt<(file<,'nolog'>)>`	Retrieve FIDs
`svf<(file<,'nolog'><,'arch'>)>`	Save FIDs in current experiment
`wft*`	Weight and Fourier transform 1D data

```
* array<(parameter<,num_steps,start,step_size)>
  dss<(start,finish<,step>)><,options>)>
  dssa<(start,finish<,step>)><,options>)>
  dssan<(start,finish<,step>)><,options>)>
  dssh<(start,finish<,step>)><,options>)>
  dsshn<(start,finish<,step>)><,options>)>
  dssn<(start,finish<,step>)><,options>)>
  ft<(<options,><'nf'><,start><,finish><,step>)>,
  ft('inverse', exp_num, expansion_factor)
  pl<(<start,finish<,step>><,'int'><,'all'><,options>)>
  wft<(<options,><'nf'><,start><,finish><,step>)>,
  wft('inverse', exp_num, expansion_factor)
```

Parameters	
`array` {string}	Parameter order and precedence
`ho` {number}	Horizontal offset
`il` {'y', 'n'}	Interleave arrayed and 2D experiments
`vo` {number}	Vertical offset

also be arrayed. To perform two experiments in which the decoupler is off in one case and on in the other, for example, you can use `dm='n','y'`.

Not all parameters can be arrayed. Non-arrayable acquisition parameters include processing parameters, display parameters, and any parameter that changes the number of data points to be acquired, such as `np`, `sw`, `dp`, and `at`.

To display the values of the arrayed parameter, the `da` command is used. `da` displays all values of arrayed parameters if entered without an argument. If one or more parameters are listed as an argument, `da` displays only the specified parameters.

Multiple Arrays

Two or more parameters can be arrayed in an experiment. For example, an experiment to perform a series of decoupling experiments using an array of decoupler power levels and an array of decoupler frequencies might be set up with `dpwr=17,20,23` and `dof=295.1,345.6,507.2,1245.5`. In this example, *twelve* experiments are performed (i.e., three different values of decoupler power `dpwr` are used), and for each of those values, four different values of the decoupler offset `dof` are used.

Setting Array Order and Precedence

Whenever an array of one or more parameters is set up, the parameter `array` becomes important. This parameter tells the system the name of the parameter or parameters that are arrayed, and the order and precedence in which the arraying is to take place.

The string parameter `array` can have one of several forms:

- `array=''` means no parameter is arrayed (this value is two single quotation marks with no space between, not a double quotation mark).
- `array='x'` means parameter `x` is arrayed.
- `array='y,x'` means parameters `x` and `y` are arrayed, with `x` taking precedence. The order of the experiments is x_1y_1, $x_2y_{1,}$ ...x_ny_1, x_1y_2, x_2y_2, ...x_my_2 ...x_my_n, with a total of $m \times n$ experiments being performed.
- `array='x,y'` means parameters `x` and `y` are arrayed, with `y` taking precedence. The order of the experiments is x_1y_1, x_1y_2,...x_1y_n, x_2y_1, x_2y_2,...x_2y_n,...x_my_n, with a total of $m \times n$ experiments being performed.
- `array='(x,y)'` means parameters `x` and `y` are jointly ("diagonally") arrayed. The number of elements of the parameters `x` and `y` must be identical, and the order of experiments is x_1y_1, x_2y_2, ...$x_ny_{n,}$ with `n` experiments being performed.

As you enter one or more arrayed parameters, `array` is automatically set for you. Only if you want to change the order or precedence is it necessary to enter `array` directly.

Interactively Arraying Parameters

Separate from the `array` *parameter* is the `array` *macro*. If you enter the `array` macro without an argument, an interactive mode is started in which you are asked for the following information, in this order:

- The name of the parameter to be arrayed.
- The number of values of the parameter.
- The starting value.
- The magnitude of the difference between elements in the array.

Using the information you provide, an arrayed parameter is set up. The restrictions are that only numeric parameters can be arrayed and all values of the array must satisfy the limits of the parameter.

Entering `array` with a parameter name as an argument, (e.g., `array('pw')`) still starts an interactive mode but the program only asks for the remaining three items of information.

If you enter the macro with all four pieces of information as arguments (in this order—parameter name, number of steps, starting value, and step size), `array` bypasses the interactive mode completely. For example, entering `array('tof',5,1000,-50)` sets the `tof` parameter to have 5 elements with the values in the order 1000, 950, 900, 850, 800.

Resetting an Array

Once an array is created, it is possible to change the value of a single element of the array by typing, for example, `pw[2]=11.3`, where the `2` enclosed in brackets indicates which element of the array to modify (array elements are counted starting at 1).

To reset an arrayed parameter to a single value, enter a single value for the parameter (e.g., `pw=10`). The `array` parameter is automatically modified to reflect this change.

Array Limitations

Regular multiple arrays can include up to 20 parameters, each of which can be a simple parameter or a diagonal array (a set of parameters), which can include up to 10 parameters. The total number of elements of all arrays is essentially unlimited (2^{32}–1).

Acquiring Data

Once any parameter is an array, entering `go` (or related commands and macros) generates not just one, but an entire array of spectra. These spectra can then be examined either individually or as a group, as described below.

Autogain cannot be used in an arrayed experiment. You can either use `gain='y'`, which sets the gain to the previously determined value, or set `gain` equal to a fixed value.

Arrayed acquisitions can be interleaved, in which a part of each experiment is done in turn rather than starting and finishing each experiment sequentially. The interleave function is controlled by the parameter `il`.

- If `il='y'`, experiments are interleaved. `bs` transients are performed for each member of the array, followed by `bs` more transients for each member of the array, and so on until `nt` transients are collected for each member of the array. Thus, `il` is relevant only if `bs` (block size) is less than `nt` (number of transients).
- If `il='n'`, all transients are acquired for the first experiment in the array, then all transients for the second experiment, etc.

Processing

The command `ft` or `wft` is used to transform all of the spectra. Both commands take the same arguments and options:

- `'acq'` does not transform elements that have already been transformed.
- `'nodc'` does not perform FID drift correction.
- `'nods'` prevents an automatic spectral display (sane as `ds` command).
- `'zero'` zeroes the imaginary channel of the FID before Fourier transform.

Phasing can be done on any spectrum. Only one set of phase correction parameters exists, so all spectra have the same phase at any one time (although the phase can of course be changed when examining different spectra).

Display and Plotting

The command `ds(index)` displays interactively the requested spectrum from the array. The index can have one, two, or three numbers, depending on the dimensionality of the spectral array. Spectra are always scaled according to the number of completed transients `ct`; if `nt` is arrayed (`nt=1,2,4,8`), each spectrum is scaled by its *own* `ct`.

Other spectra display commands are `dss`, `dssn`, `dssa`, `dssan`, `dssh`, `dsshn` and `dssl`. These are not interactive like the `ds` command. They display stacked spectra in which each spectrum is offset with respect to the previous spectrum. The order of stacking can be left to right, right to left, top to bottom, or bottom to top, depending on whether the horizontal offset (`ho`) and vertical offset (`vo`) parameters are positive or negative. Some of these commands set `ho` and `vo` automatically.

The spectra display commands function as follows:

- `dss` displays stacked spectra using the current values of `ho` and `vo` to set the order of stacking.
- `dssn` displays stacked spectra the same as `dss`, but the graphics window is not erased before starting the display. This allows composite displays of many spectra to be created.
- `dssa` displays stacked spectra automatically (i.e., `vo` and `ho` are automatically adjusted to fill the screen in a lower left to upper right presentation).
- `dssan` displays stacked spectra automatically the same as `dssa`, but the graphics window is not erased before starting the display.
- `dssh` displays stacked spectra horizontally (i.e., `vo` is set to zero and `ho` is adjusted to fill the screen from left to right).
- `dsshn` displays spectra horizontally the same as `dssh`, but the graphics window is not erased before starting the display.
- `dssl` displays a label for each element in a set of stacked spectra. The label is an integer value starting with 1 and extending up to the number of spectra in the display.

The command `pl` plots stacked spectra with the same format as displayed by `dss`.

The argument syntax `<(start,finish<,step>)><,options>)>` is used by the `dss` command, variants of `dss`, and by the `pl` command. The arguments are the following:

- `start` is the index of the first spectra when displaying multiple spectra. It is also the index number of a particular trace to be viewed when displaying arrayed 1D spectra or 2D spectra.
- `finish` is the index of the last spectra when displaying multiple spectra. Because the parameter `arraydim` is automatically set to the total number of spectra, it can be used to set `finish` to include all spectra.
- `step` is the increment for the spectral index when displaying multiple spectra. The default step is 1.
- `options` can be any of the following:

  `'all'` is a keyword to display all of the spectra.

  `'int'` is a keyword to only display the integral, independently of the value of the parameter `intmod`.

  `'top'` or `'side'` are keywords that cause the spectrum to be displayed either above or at the left edge, respectively, of a contour plot. This assumes that the parameters `sc`, `wc`, `sc2`, and `wc2` are those used to position the contour plot. This option does not apply to `dssa`, `dssan`, `dssh`, or `dsshn`.

  `'dodc'` is a keyword for all spectra to be drift corrected independently.

  `'red'`, `'green'`, `'blue'`, `'cyan'`, `'magenta'`, `'yellow'`, `'black'`, and `'white'` are keywords that select a color. This option does not apply to `dssa`, `dssan`, `dssh`, `dsshn`, or `pl`.

  `'pen1'`, `'pen2'`, `'pen3'`, etc. specify a pen number on a plotter. This option does not apply to `dss` or any of its variants.

Saving and Retrieving

The commands `rt` and `svf` retrieve and save arrayed data, just like single 1D data sets. The entire data, consisting of a number of FIDs, is stored and retrieved together as a single file.

Pulse Width Calibration Step-by-Step

To illustrate using arrays, note how the following steps perform a pulse width calibration:

1. Set up parameters and obtain a normal spectrum of any sample. For best results, one or more intense signals should appear near the center of the spectrum.
2. Enter **pw=5**. You can use some other small value if you wish.
3. Enter **nt=1**.
4. Obtain a spectrum and phase it properly. Set **d1** to 5*T_1.
5. Enter **pw=5,10,15,20,25,30 ai go**.

   You can use some other set of suitable values for the pw array.
6. After the experiment finishes acquisition, enter **wft dssh**.
7. Find the experiment where the signal goes through its 180° or 360° null. Enter **da** to remind yourself of the values of the pw array.
8. To reset the array, enter **pw=10**.

1.3 T_1 and T_2 Analysis

One relatively common form of arrayed experiment is the inversion-recovery T_1 experiment. In this experiment, the nuclei are allowed to relax to equilibrium (d1), then inverted with a 180° pulse (p1), given a variable time to return to equilibrium (d2), and finally given a monitoring 90° pulse (pw) to measure their peak height as a function of d2. Under most circumstances, the behavior of the peak heights as a function of d2 will be exponential, and this exponential time is the T_1.

Table 3 lists commands and parameters associated with the T_1 and T_2 analysis.

Table 3. T_1 and T_2 Analysis Commands and Parameters

Commands	
autoscale	Resume autoscaling after limits set by scalelimits
dels(index1<,index2,...>)	Delete spectra from T_1 or T_2 analysis
dot1*	Set up a T_1 experiment
expl<(<options,>line1,line2,...)>	Display exponential or polynomial curves
fp*	Find peak heights
pexpl*	Plot exponential or polynomial curves
scalelimits*	Set limits for scales in regression
t1	T_1 exponential analysis
t1s	T_1 exponential analysis with short output table
t2	T_2 exponential analysis
t2s	T_2 exponential analysis with short output table

```
* dot1<(minimum_T1_estimate,maximum_T1_estimate,time)>
  fp<(<'phase',><index1,index2,...>)>
  pexpl<(<options,>line1,line2,...)>
  scalelimits(x_start,x_end,y_start,y_end)
```

Parameter	
npoint {1 to fn/4}	Number of points for fp peak search

Setting Up The Experiment

The standard two-pulse sequence is set up to perform the T_1 experiment. You can start if you wish by entering appropriate values for `p1`, `pw`, `d1`, and an array of values for `d2`.

Alternatively, you can use the `dot1` macro. `dot1` sets up all parameters to perform a T_1 experiment, including `d1`, `pw`, `p1`, `nt`, and an array of `d2` values, based on information you enter. The three arguments that can be input are the minimum expected T_1, the maximum expected T_1, and the total time in hours the experiment should take. If no arguments are provided, `dot1` prompts the user for the information.

Be sure that the parameter `pw90` is set properly and contains the correctly calibrated 90-degree pulse width, because `dot1` uses this information.

Processing the Data

Once the data is acquired, process the data as follows:

1. Enter **`wft ds(arraydim)`** to display the last spectrum (or **`ds(1)`** for a T_2 experiment to display the first spectrum).
2. Phase this spectrum properly.
3. Select a threshold and adjust the threshold line position.
4. Enter **`dpf`** or **`dll`** to display a line list and locate lines for the system.
5. Enter **`fp`** to measure the peak height of each peak in an array of spectra. If optional line indexes are supplied to `fp` as arguments (e.g., `fp(1,3)`), only the peak heights of the corresponding lines are measured.

   The `npoint` parameter (if defined and set “on”) determines the range of data points over which the `fp` command searches for a maximum for each peak.

Analyzing the Data

T_1 and T_2 analysis is performed by the `t1` and `t2` macros, respectively. `t1` and `t2` measure relaxation times for all lines in the line listing and display an extended listing of observed and predicted peak intensities. `t1s` and `t2s` perform the same calculation as `t1` and `t2` but produce a shorter output, showing only a summary of the measured relaxation times.

The command `expl` displays exponential/polynomial curves resulting from T_1, T_2, or kinetic analysis. Optional input of line numbers as arguments allows displaying only selected lines. Similarly, the command `pexpl` plots the same curves.

The macro `autoscale` returns the command `expl` to autoscaling in which scale limits (set by `scalelimits`) are determined that will display all the data in the `expl` input file. The macro `scalelimits` causes the command `expl` to use typed-in scale limits. If no arguments are given, `scalelimits` asks for the desired limits. The limits are retained as long as an `expl` display is retained.

To delete spectra from the `t1` or `t2` analysis (or from `t1s` or `t2s`), enter `dels(index1<, index2>...)`. This command deletes the spectra selected by the indexes from the output file `fp.out` of the `fp` command used by the `t1` or `t2` analysis. Spectra can be restored by rerunning `fp`.

Exponential Analysis Menu

Most of the commands for working with T_1 and T_2 analysis are available by clicking on Main Menu button, followed by the Analyze button, and then the Exponential button. The following menu, called the Exponential Analysis menu, is displayed

This menu can also be displayed by entering the command `menu('t1t2anal')`.

T_1 Data Workup: Step-by-Step

The following procedures accomplish the same result.

Using Menus

1. From the Permanent menu, click on **Main Menu** > **File** > **Set Directory** > **Parent**

   The text output window shows a list of directories (entries with a slash as last character) and files (if any). The status window (at the top of the screen) shows the directory path you are in currently.

2. Click the **Parent** button as many times as necessary on until the message "`Directory now /`" appears in the status window.

3. Click the mouse on `vnmr/` so it turns to inverse video, then click on the **Change** button. You are now in the `vnmr` directory.

4. Click the mouse on `fidlib/` so it turns to inverse video, then click on the **Change** button. You are now in the `fidlib` directory.

5. Click the mouse on the `t1data.fid` so it turns to inverse video, then click on the buttons **Return** > **Load**.

6. Click on **Analyze** > **Exponential** > **T1 Proc** > **T1 Analysis**.

   The T_1 analysis appears in the text output window.

Using Commands

1. Enter **`rt('/vnmr/fidlib/t1data.fid')`**.
2. Enter **`wft dssh full ds(arraydim) aph`**.
3. Click on **Next** > **Th**. Use the left mouse button to set the threshold.
4. Enter **`dll fp t1 center expl`**.

1.4 Kinetics

The arraying capability of the VNMR software provides for the acquisition of data for the study of kinetics. Table 4 lists commands and a parameter for kinetics analysis.

Setting Up the Experiment

Usually, the best procedure is to array the preacquisition delay parameter `pad`. For example, if `pad=0,3600,3600,3600,3600`, the system acquires the first spectrum immediately (`pad[1]=0`), waits 3600 seconds (`pad[2]=3600`), acquires the second

Table 4. Kinetics Analysis Commands and Parameters

Commands	
`kind`	Kinetics analysis, decreasing intensity
`kinds`	Kinetics analysis, decreasing intensity, short form
`kini`	Kinetics analysis, increasing intensity
`kinis`	Kinetics analysis, increasing intensity, short form
Parameter	
`pad` {number, in sec}	Preacquisition delay

spectrum, waits another 3600 seconds, etc. Because 3600 seconds is 1 hour, this inserts a wait of one hour between acquisitions. After all the spectra have been obtained, they are processed much like T_1 or T_2 data.

Processing the Data

If the signal decreases exponentially with time, the output is matched to the equation `I=A1*EXP(-T/TAU)+A3`. The analysis is done by the macro `kind`, or by macro `kinds` if a short output table is desired.

If the signal increases exponentially with time, the output is matched to the equation `I=-A1*EXP(-T/TAU)+A3-A1` with analysis done by the macro `kini`, or by the macro `kinis` for a short output table.

Kinetics Step-by-Step

The following steps are typical in processing a kinetics experiment:

1. Enter **`wft dssh full ds aph`**.
2. Click on **Next** > **Th**. Use the left mouse button to set the threshold.
3. Enter **`dll fp`**.
4. Enter **`kind`**, **`kini`**, **`kinds`**, or **`kinis`**, as appropriate (see Table 4).
5. If desired, adjust `sc`, `wc`, `sc2`, and `wc2` by entering **`center`** or **`full`**.
6. Enter **`expl`**.

1.5 Diffusion Experiments

Software for diffusion measurements using a pulsed Z-gradient probe is an optional feature of Varian NMR systems. This section describes the pulsed gradient experiment and its analysis. Table 5 summarizes the commands used in diffusion experiments. See the *PFG installation* manual concerning installing the Performa PFG module, and see the *VNMR and Solaris Software Installation* manual for instruction on loading the diffusion software.

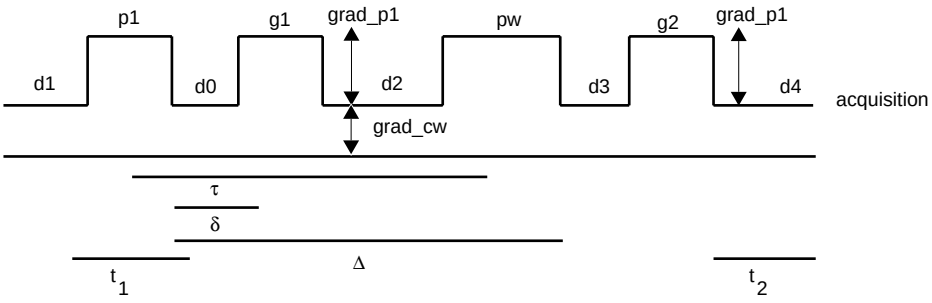
Pulsed Gradient Experiments

The pulse sequences diagrammed in Figure 1 illustrate the two pulsed gradient experiments. These experiments are fully described in the literature by Stejskal and Tanner (Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, ***42***, 288-292, and Tanner, J. E. *J. Chem. Phys.* **1970**, ***52***, 2523–2526).

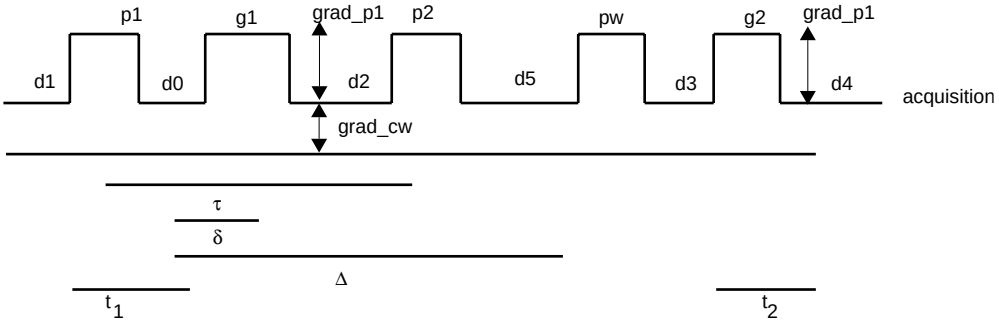
Table 5. Diffusion Analysis Commands

Commands	
`analyze*`	Generalized curve fitting
`expfit*`	Make least-squares fit to polynomial or exp. curve (UNIX)
`expl`	Display exponential or polynomial curves
`expladd(integral_region)`	Add another diffusion analysis to current display
`pexpladd(integral_region)`	Add another diffusion analysis to current plot
`pge`	Convert parameter set to PGE pulse sequence
`pge_calib`	Calibrate gradient strengths for PGE pulse sequence
`pge_data(array_index)`	Extract data from single element of PGE pulse sequence
`pge_output`	Output results from PGE pulse sequence
`pge_process`	Automated processing of data from PGE pulse sequence
`pge_results*`	Calculate diffusion constant for integral region
`pge_setup<('no')>`	Set up gradient control parameters for PGE pulse sequence

```
* analyze('expfit",xarray,<,option,option,...>)
  expfit options <analyze.inp >analyze.list (UNIX)
  pge_results(integral_region<,reference_region>)
```



pge — Pulsed Gradient Experiment (stim='n')



pge — Stimulated Echo Experiment (stim='y')

Figure 1. PGE Pulse Sequences

A pulse sequence named PGE and its associated help file are provided. A parameter set `/vnmr/parlib/pge` is used as the master parameter set. Table 6 lists the new gradient control parameters.

Table 6. Gradient Control Parameter Set

Name	*Description*	*Units*	*Used In*
`stim`	select normal echo or stimulated echo experiment	`'n','y'`	pulse sequence
`d0–d5`	acquisition delays	sec	pulse sequence
`p1,p2,pw`	RF pulse widths	msec	pulse sequence
`g1,g2`	gradient pulse widths	msec	pulse sequence
`grad_cw`	continuous-wave gradient amplitude	gauss/cm	pulse sequence
`grad_p1`	pulsed gradient amplitude	gauss/cm	pulse sequence
`grad_p2`	pulsed gradient amplitude	gauss/cm	pulse sequence
`dac_cw`	continuous-wave gradient amplitude	dac units	pulse sequence
`dac_cw`	pulsed gradient amplitude	dac units	pulse sequence
`dac_cw`	pulsed gradient amplitude	dac units	pulse sequence
`g_max`	maximum gradient value		macro `pge_setup`
`g_min`	minimum gradient value		macro `pge_setup`
`g_steps`	number of gradient values		macro `pge_setup`
`g_array`	type of gradient array		macro `pge_setup`
`nt_array`	type of `nt` array		macro `pge_setup`
`nt_first`	first nt value		macro `pge_setup`
`nt_fract`	fractional nt increment		macro `pge_setup`

The parameters `p2` and `d5` are only used for the stimulated echo experiment (`stim='y'`). The parameter `p1` defines the first rf pulse, `pw` defines the last rf pulse. Delay `d1` occurs before the first rf pulse.

With the current pulse sequence, `grad_p1` defines the amplitudes of both pulsed gradients: `g1` and `g2`. Unused parameters `grad_p2` and `dac_p2` are provided if you wish to modify the pulse sequence to use different gradients. These parameters are unused by the `pge` pulse sequence that is provided.

The PGE pulse sequence checks the average power output of the gradient coils for a safe value. Maximum value is 100 gauss/cm for a continuous wave gradient. This value is hard-coded into the pulse sequence by defining a numerical value in the pulse sequence for the variable `dutycycle`. The value is calculated from the following expression:

$$\texttt{dutycycle} = \sqrt{\frac{(\texttt{grad\_sw})^2 \times \texttt{time} + (\texttt{grad\_p1})^2 \times (\texttt{g1} + \texttt{g2})}{\texttt{time}}}$$

where `time=g1+g2+d0+d1+d2+d4+d5+p1+p2+pw+at`.

The user should change this to fit the needs of a specific probe. Comments in the pulse sequence file `/vnmr/psglib/pge.c` contain more details about the sequence.

Pulsed Gradient Experiment Setup

There are two ways to retrieve a set of parameters for the pulsed gradient experiment:

- Enter `rt('/vnmr/parlib/pge')`. This command returns a complete parameter set into the active experiment.
- Enter `pge(macro_name)`. This appends the required parameters to those already present in the active experiment.

For the data from a diffusion experiment to be analyzed properly, it is necessary to define integral regions in the Fourier transformed spectra. The simplest way to define these regions is to run the pulsed gradient experiment as a simple echo experiment (`grad_p1=0`): first, to check that the values of τ and 2τ start the data acquisition at the top of the echo, and second, to define integral regions in the experiment where the pulsed field gradient experiment will be subsequently done. The phase parameters (`rp` and `tp`) should also be determined for this spectrum. If data acquisition was started at the top of the echo, the `lp` phase parameter should be zero. The easiest way to define integrals is by using the RESETS button of the `ds` command.

Once the parameters are in the experiment, those associated with gradient control can be set by entering `pge_setup`. This macro has a single optional argument to turn off interactive questioning; any argument can suffice (e.g., `pge_setup('no')`).

The macro `pge_setup` performs the following three tasks.

1. Sets the gradient (`grad_p1`) array.

   If the interactive mode is used, `pge_setup` prompts for the values of `g_max`, `g_min`, `g_steps`, and `g_array`. These parameters are used to calculate the gradient amplitude array. Manual override is provided by typing in each value. The value of `g_array` can be set to `'linear'` if the gradient values are equally spaced between `g_min` and `g_max`, or set to `'square'` if the square of the gradient values is equally spaced between the square of `g_min` and the square of `g_max`.

2. Sets the number of transients (`nt`) array.

   If the interactive mode is used, `pge_setup` prompts for the values of `nt_array` and `nt_first`. The values of `nt_array` are `'same'` and `'fraction'`. The parameter `nt_first` is used to set to the value of the first `nt` array element. If `'same'` is selected, all elements of the `nt` array are set to `nt_first`. If `'fraction'` is selected, `nt_fract` is set so that elements of the `nt` array are calculated according to the equation:

   $nt_i = nt\_first \times [1 - i \times (1 - nt\_fract) / g\_steps]^2$

   Manual override is provided by typing in each value.

3. Does necessary housekeeping.

   The `array` parameter is set so that `nt` and `dac_p1` form a “diagonal array.” The `time` macro is executed to display the experiment duration. The average gradient level of the particular parameter combination is checked when acquisition is attempted. The `wexp` parameter is set to `'pge_process'` to perform appropriate data processing at the end of the experiment.

Other acquisition parameters can be altered by typing in new values. Once a “good” set of parameters is entered, it may be saved for future recall with the `svp` (save parameters) command. The automatic processing of the diffusion data following data acquisition is initiated with the `au` command if `wexp` is set to `'pge_process'`.

Gradient Calibration

Calibration constants, which relate DAC values (in DAC units) to resulting values (in gauss/cm), are stored in `conpar` with names `grad_cw_coef` and `grad_p_coef`. These coefficients are in units of (gauss/cm)/(dac unit). The DAC values are whole numbers while gradient values are real numbers that may contain fractional parts.

Whenever the `dac_x` or `grad_x` parameters are changed, where `x` is `cw`, `p1`, or `p2`, macros are available to adjust the dependent parameter, taking into account possible minimum and maximum values and housekeeping in case the parameters are arrayed. The mathematical relationships are defined as follows:

- When `dac_x` is changed: `grad_x=dac_x*grad_x_coef`
- When `grad_x` is changed: `dac_x=grad_x/grad_x_coef`
  `grad_x=dac_x*grad_x_coef`

The second step taken, when `grad_x` is changed, is necessary because the calculation of `dac_x` is rounded to the nearest integer, which necessitates that `grad_x` then be recalculated so that it corresponds to `dac_x`.

A macro `pge_calib` is provided to assist in the calibration of acquisition parameters that control the gradient power levels (i.e., the DAC values). After phasing and selecting the integral region of the standard sample, run a pulsed gradient experiment and process data with the `pge_process` macro. The calculated diffusion constant is displayed in the text window. Then run the `pge_calib` macro to recalculate the coefficient. This macro resets the set of data, followed by processing with the `pge_process` macro, and should now give the diffusion constant that was selected with the `pge_calib` macro. After running `pge_calib`, run `pge_setup` again to calculate DAC values with the new coefficient.

Data Reduction

The `pge_process` macro performs several tasks (see Figure 2), calling macros as appropriate.

1. Transforms the data with the specified weighting function.
2. For each array element (i.e., spectrum), writes into a text file the following information: the gradient value for that spectrum, the integral amplitudes, and the spectral parameters needed to reproduce the data.

   The file is stored in the current experiment directory and its name reflects which element of the array this information pertains to (e.g., `info_1` to `info_n`). The macro `pge_data` is called by `pge_process` to do this for each array element.

   The `pge_data` macro has the element number passed as an argument. This feature allows an operator to manually adjust the spectral parameters for a single element and then invoke the `pge_data` macro to update the raw information in the appropriate text file.

   A sample information file for the second element of an array is the following:

```
Gradient amplitude for spectrum 2 is 63.9715
Spectral parameters:
rp= 344.604 lp= 0 lvl= 0 tlt= 0
sp= –5000 wp= 10000 is= 691.5 ins= 1 fn= 32768
2 Integral Regions          Value
7440186      –46.5117       4.58914
–325.582     –1604.65       36.8508
```

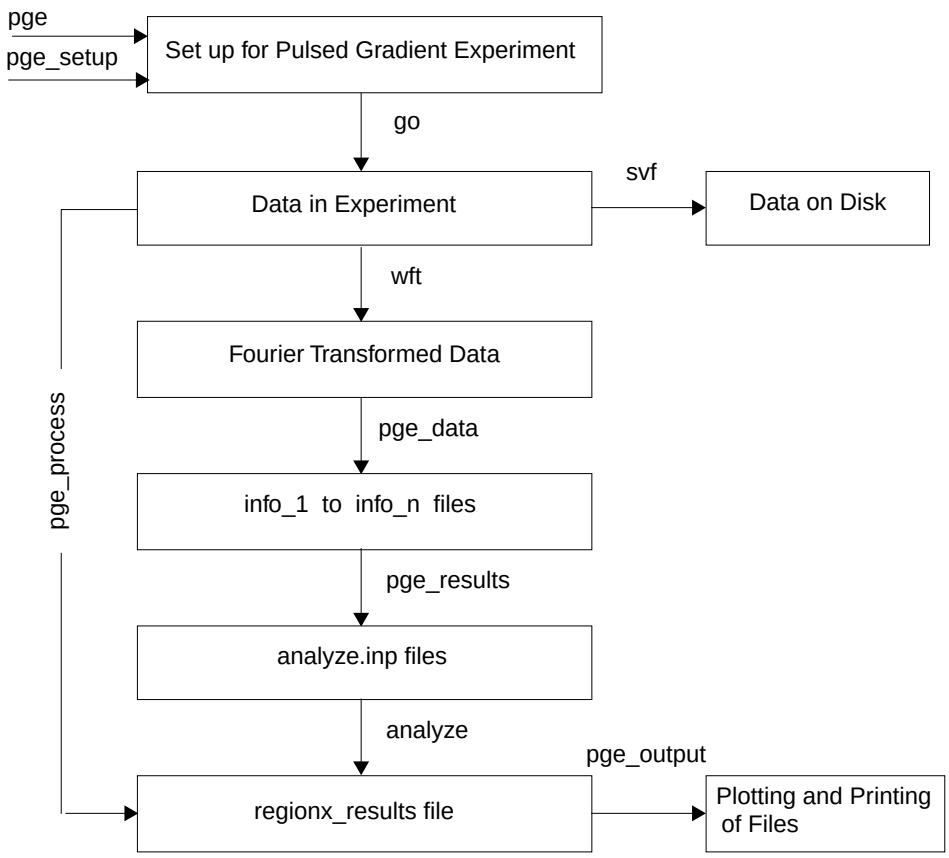


Figure 2. Data Processing Macros Flowchart

To manually correct the data from a single element of the data array, the following sequence can be used: select the desired array element with `ds` (e.g., `ds(5)`); manually adjust the phase or drift corrections; then, type `pge_data(5)` to write the new integral information into the `info_5` text file.

3. The `pge_process` macro calls the `pge_results` macro. `pge_results` collects the necessary information from the `info_n` files and constructs a text file `analyze.inp` that is used to calculate the diffusion coefficient. `pge_results` requires a single argument that indicates which integral region to use when recalculating the diffusion coefficient with the information from `info_n` files.

   The `pge_process` macro calls `pge_results` once for each of the defined integral regions. Figure 3 is a sample `analyze.inp` text file.

```
List of 8 x-y data
pairs
diffusion data for   \
integral region 2
Grad*Grad    ln(Amp)
0            4.23277
4092.35      3.60688
8192.57      3.01594
12305.2      2.37857
16369.4      1.72547
20619.8      1.13322
```

Figure 3. Sample `analyze.inp` File

The diffusion coefficient is calculated using Equation 1:

$$A_i = A_0 \exp -\gamma^2 D\left\{\frac{2}{3}t^3 \times \texttt{grad\_cw}^2 + \delta^2\left(\Delta - \frac{1}{3}\delta\right)\texttt{grad\_p1i}^2 - \delta\left[(t1^2 + t2^2) + \delta(t1 + t2) + \frac{2}{3}\delta^2 - 2\tau^2\right]\texttt{grad\_p1i} \times \texttt{grad\_cw}\right\}$$

where A_i is the observed integral value of an NMR resonance for the *i*th element of the gradient array. A_0 is the integral value of an NMR resonance just after the first 90° pulse in the pulse sequence. Δ is the self-diffusion coefficient. In the case of the two-pulse echo sequence, the variables τ, δ, Δ, *t1*, and *t2* (refer to Figure 1) are calculated from the pulse sequence variables as follows:

$$\tau = \frac{\texttt{(p1)}}{2} + \texttt{d0} + \texttt{g1} + \texttt{d2} + \frac{\texttt{pw}}{2}$$

$$\delta = \texttt{g1}$$

$$\Delta = \texttt{g1} + \texttt{d2} + \texttt{pw} + \texttt{d3}$$

$$t1 = \frac{\texttt{p1}}{2} + \texttt{d0}$$

$$t2 = \texttt{d4}$$

In the case of the stimulated-echo sequence, the equation for Δ is as follows:

$D = \texttt{g1} + \texttt{d2} + \texttt{p2} + \texttt{d5} + \texttt{pw} + \texttt{d3}$

Equation 1 can be recast as the following, which becomes Equation 2:

$$ln(A_i) = ln(A_0) + D \times C0 + D \times C1 \times \texttt{grad\_p1}i + D \times C2 \times \texttt{grad\_p1}i \times \texttt{grad\_p1}i$$

where:

$$C0 = \frac{2}{3}\gamma^2\tau^3 \text{grad\_cw}^2$$

$$C1 = \gamma^2\delta\left[(t1^2 + t2^2) + \delta(t1 + t2) + \frac{2}{3}\delta^2 - 2\tau^2\right] \times \text{grad\_cw}$$

$$C2 = -\gamma^2\delta\left(\Delta - \frac{1}{3}\delta\right)$$

The fitting program `analyze` accepts two arguments that instruct it to perform a polynomial fit. The selection of which polynomial to fit depends on whether `grad_cw` is zero. If it is zero, the second and third terms of Equation 2 vanish and a first-order polynomial $y = c0 + c1 \times x$ is used where:

$y = ln(A_i)$
$c0 = ln(A_0) + D \times C0$
$c1 = D \times C2$
$x = \texttt{grad\_p1}i$

Otherwise, a second-order polynomial $y = c0 + c1 \times x + c2 \times x \times x$ is used, where:

$y = 1n(A_i)$
$c0 = 1n(A0) + D \times C0$
$c1 = D \times C0$
$c2 = D \times C2$
$x =$ `grad_p1`i

Another argument of `analyze` is the complete name of a text file (`analyze.inp`) that contains the *x*–*y* data pairs. The output of this calculation is written into a text file in the current experiment directory. The name of this text file reflects the integral region on which the analysis was performed. This name has the form `regionx_results`, where the `x` is the integral region number. Using the experimental delays, `grad_cw` and `gamma`, the `pge_results` macro calculates the diffusion constant and the time-zero integral amplitude from the fitting parameters `c0`, `c1`, and `c2`. The results of these calculations are appended to the text file that contains the least-squares analysis results.

The diffusion coefficients of both components of a two component mixture can be calculated assuming the following condition is met. It is possible to find one integral region where the NMR resonance is due to only one component of the mixture. The diffusion coefficient is calculated using that integral region with the processing already described. For integral regions, where the NMR intensity results from both components of a two component mixture, Equation 1 transforms to the following, which becomes Equation 3:

$$A_i = a0 \times exp[D \times (C0 + C1 \times \texttt{grad\_p1}i + C2 \times \texttt{grad\_p1}i \times \texttt{grad\_p1}i)] + a2 \times exp[a1 \times D \times (C0 + C1 \times \texttt{grad\_p1}i + C2 \times \texttt{grad\_p1}i \times \texttt{grad\_p1}i)]$$

The diffusion coefficient *D* is available from the separate reference integral region. The constants *C*0, *C*1, and *C*2 are defined in Equation 2. The fitting parameters are *a*0, *a*1, and *a*2. In order to perform the non-linear least squares analysis of Equation 3, the `pge_results` macro is supplied with two arguments (e.g., `pge_results(1,3)`). The first argument is the region on which to perform the analysis (just as for the single-component analysis case) and the second argument is the integral region used to get the value of *D*. The fitting parameter a0 corresponds to the time-zero integral amplitude of the reference component; *a2* corresponds to the time-zero amplitude of the other component; *a1* corresponds to the ratio of the two diffusion coefficients.

Data Display

The macro `pge_output` prints the experimental parameters and the results of the diffusion calculations. It also prints graphs of the line fitting results and the spectra.

As with any printing operation, the `pge_output` macro calls `printon`, does a `cat` of the `regionx_results` files, and then calls `printoff`. The plotting is done with the `pexpl` command. The analogous `exp1` command displays graphs on the screen.

Two macros are supplied that add the results of the separate calculation to an existing graph. These are called `expladd` and `pexpladd`, for graphics display and plotting, respectively. Each requires a single argument that specifies the number of the region whose results are to be added to the existing plot or graph.

To plot or display the results of a two-component analysis, the commands `pexpl` and `expl`, respectively, are provided. For example, to plot the results of a two-component analysis, enter `pexpl('square','log')`. This command makes a plot of the square of the gradient versus the natural logarithm of the amplitude.

Variations on the pge Pulse Sequence

In addition to the basic pulse sequence pge for diffusion measurements, there are pulse sequence variations on pge:

pgeramp	Ramps gradients, unlike pge. Once the pge parameter set has been recalled, set seqfil='pgeramp' to execute pgeramp. Use this pulse sequence when probe impedance is highly mismatched to the gradient amplifier output. pgeramp determines ramp length (defined in µs). When executed, this pulse sequence determines the number of steps in ramping the gradient based on the value of tramp (default value is 200 µs) and the gradient strength. In arrayed series of gradients, lower gradients have fewer steps and higher gradients have more steps.
g2pulramp	Analogous to g2pul except that the gradients are ramped and ramp time is determined by tramp. g2pulramp is executed by setting seqfil='g2pulramp'. It determines the number of steps in ramping the gradient based on the value of tramp (the default value is 200 µs) and the gradient strength.

DOSY Experiments

The DOSY (**D**iffusion **O**rdered **S**pectroscop**Y**) application separates the NMR signals of mixture components based on different diffusion coefficients. Generally speaking, DOSY increases the dimensionality of an NMR experiment by one. In 2D DOSY the initial diffusion weighted NMR spectra are one-dimensional; adding diffusion weighting to a 2D NMR experiment such as COSY or HMQC gives 3D DOSY spectra.

The DOSY analyzes involves two steps. These steps are executed by the dosy macro.

1. Set up and acquire a DOSY spectrum.
2. Determine the diffusion coefficients for each line (or cross-peak) in the spectrum. Take line (or cross-peak) positions and diffusion coefficients and show the results in a DOSY plot.

Table 7 shows the available tools for DOSY.

DOSY Pulse Sequences

Previous DOSY pulse sequences used an unhelpful choice of parameter names. These names have been corrected, but compatibility with old data has largely been maintained. The dosy macro attempts to identify the relevant information from the parameters and the pulse sequence name; if it fails, dosy starts a dialog asking for three pieces of required information:

- The width of the gradient pulse(s) used for dephasing before diffusion.
- The diffusion delay between dephasing and rephasing.
- For bipolar sequences, the time between the positive and negative gradient pulses.

New sequences always start with “D” and are supplied with this version of the DOSY software. The sequences calculate the time portion of the exponent governing diffusional attenuation, storing the calculation as dosytimecubed, and the Larmor frequency of the diffusing spins, storing that calculation as dosyfrq.

The macros bppste and showdosy are obsolete in VNMR 6.1C.

Table 7. Tools for the DOSY Experiment

Command	Function
`cleardosy`	Delete any temporarily saved data in the current (sub) experiment.
`ddif`	Synthesize and display DOSY plot.
`Dgcstecosy`	Set up parameters for the Dgcstecosy.c pulse sequence.
`Dgcstehmqc`	Set up parameters for the Dgcstehmqc.c pulse sequence.
`DgsteSL`	Set up parameters for the DgsteSL.c pulse sequence.
`Doneshot`	Set up parameters for the Doneshot.c pulse sequence.
`dosy`	Process DOSY experiments.
`Dbppste`	Set up parameters for the Dbppste.c pulse sequence.
`Dbppsteinept`	Set up parameters for the Dbppsteinept.c pulse sequence.
`fbc`	Apply baseline correction for each spectrum in the array.
`fiddle*`	Perform reference deconvolution.
`makedosyparams`	Create DOSY-related parameters (called by setup macros).
`makeslice`	Synthesize 2D projection of a 3D DOSY spectrum in diffusion limits.
`redosy`	Restore the previous 2D DOSY display from the subexperiment.
`setup_dosy`	Start dialog to set up gradient levels for DOSY experiments.
`sdp`	Show diffusion projection.
`setgcal`	Set the gradient calibration constant.
`showoriginal`	Restore the first 2D spectrum in a 3D DOSY experiment.
`undosy`	Restore the original 1D NMR data from the subexperiment.

`* fiddle(option<,file><,option<,file>><,start><,finish><,increment>)`

General Considerations

The DOSY experiments are probably the most demanding gradient sequences in NMR. In conventional coherence-pathway-selected-experiments, you can optimize the experimental conditions for a given gradient setting. However, in DOSY, very often the whole scale of available gradient power is used and the high-resolution NMR conditions still must be maintained. Convection, i.e., moving liquid columns along the sample axis (primarily due to temperature gradients), does not seriously hurt coherence-pathway-selected-experiments (apart from the obvious intensity losses), but, it can make the DOSY analysis completely useless.

DOSY pulse sequences use the gradient stimulated echo element (or one of its modifications), shown in Figure 4.

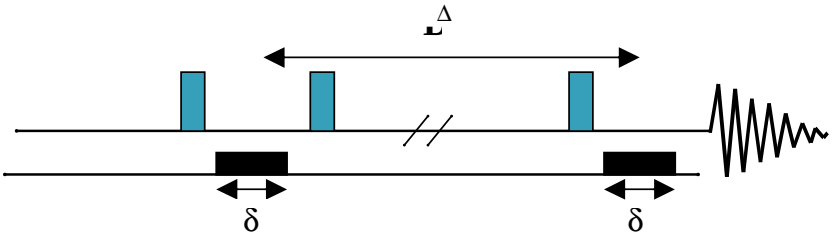


Figure 4. Gradient Stimulated Echo Element

In the DOSY experiments the strength of the diffusion encoding gradient is arrayed and the diffusion coefficients are calculated according to the Stejskal-Tanner formula:

$$S(G_{zi}) = S(0)\exp(-D_i\gamma^2\delta^2(G_{zi})^2(\Delta - \delta/3))$$

where $S(G_{zi})$ and $S(0)$ are the signal intensities obtained with the respective gradients strengths of G_{zi} and 0. D is the diffusion coefficient. γ is the gyromagnetic constant, δ is the gradient pulse duration and Δ is the diffusion delay.

The formula itself provides valuable hints on how to set DOSY-related parameters in different pulse sequences.

- $(\gamma*\delta*G_{zi})^2$ is the gradient pulse area.

  Nuclei with higher γ are more sensitive to diffusion than the low-γ nuclei. If possible, observe ^{1}H, ^{19}F, or at least do the diffusion encoding step on the high-γ nucleus. See "Dbppsteinept (DOSY Bipolar Pulse Pair Stimulated Echo INEPT) Experiment," page 54.

  Shaping a gradient dramatically reduces its phase encoding efficiency. Although VNMR software can support gradient shaping on UNITY*plus* or UNITY*INOVA* spectrometers, no advantages are expected from using shaped gradients.

- δ is the gradient pulse duration.

  During δ (and the subsequent gradient stabilization delay, `gstab`) the magnetization is transverse and subject to T_2 relaxation and homonuclear J-evolution. Do not use long δ values in the presence of large homonuclear couplings or short T_2 relaxation times ($\delta << T_2$ or 1/J).

- G_z is the gradient strength. Use as high values as possible provided high-resolution NMR conditions are still maintained (no phase, amplitude, and line shape distortions).

- Δ is the diffusion delay. Convection can always be an unwanted competitor to diffusion and T_1 relaxation attenuates the signal intensities. Do not use unnecessarily long diffusion delays ($\Delta < T_1$).

Some of the previous recommendations might seem contradictory. Of course, in real cases you need to find an acceptable compromise between them.

The separation efficiency in the diffusion domain is determined by the accuracy of the measured diffusion coefficients. DOSY does not necessarily intend to get absolute diffusion coefficients (in mixtures, it is difficult to discuss "absolute" numbers anyway); the relative differences in the D values might be adequate for separation.

Note: Changing the solvent of a DOSY mixture might change the diffusion coefficients and the separation power of the method. The solvent might play a similar role in DOSY as the different columns in HPLC.

Diffusion coefficients errors can either be statistical or systematic. The most obvious source of statistical errors is inappropriate signal-to-noise ratio; therefore in DOSY experiments, relatively high S/N values must be reached even with the strongest phase encoding gradients. Systematic errors are primarily caused by instrumental imperfections (such as gradient nonlinearity over the active sample volume, phase distortions, changes in experimental lineshape as a function of gradient amplitude). Systematic errors can be minimized by careful pulse sequence design (see *Magn. Reson. Chem.* (1998), **36:** 706) and by adding a suitable internal reference to the sample (a component producing a strong, well isolated singlet peak in the spectrum) suitable for reference deconvolution (FIDDLE) when processing DOSY.

When setting up DOSY experiments, consider the following recommendations:

- Be sure that the `probe` parameter is set to the probe you intend to use and `Probegcal` has the right value (the setup macros extract the gradient strength, `gcal`, from the probe file and store it in the local parameter DAC_to_G.) Pulse power levels and pw90 values are also read from the probe calibration file.
- Set z0 precisely on resonance and carefully adjust the lock phase. Incorrect adjustment might cause progressive phase errors with increasing gradient power.
- Do not spin the sample.
- Use an adequate number of data points for proper spectral digitization.
- When running long experiments, use interleaved acquisition.
- To minimize temperature gradients (and convection), avoid using extreme (low and high) temperatures. For solutions with very low viscosity, you might prefer to completely switch off the VT controller.
- If you can find a substance suitable for reference deconvolution, add it to the mixture before running DOSY (in proton spectra, TMS might be an ideal candidate).

2D-DOSY Spectroscopy

The current DOSY package includes four 2D DOSY sequences:

- Dbppste
- DgcsteSL
- Doneshot
- Dbppsteinept

Setting Up 2D-DOSY Experiments

1. Start setting up any of the four experiments by recording a normal s2pul spectrum on the nucleus to be observed.
2. Calibrate (or check) pulse widths if necessary.
3. Before calling the `setup` macro, which always has the same name as the pulse sequence itself, reduce the spectral window to the region of interest.
4. Each sequence has a parameter called `delflag`. By setting `delflag='y'`, the actual DOSY sequence is activated. Setting `delflag='n'` enables you to go back to the basic s2pul (Dbppste, DgcsteSL, Doneshot) or INEPT (Dbppsteinept) sequence without changing the experiment or the parameter set.
5. In all of the sequences, the phase encoding gradient duration is defined by the `gt1` parameter (the total defocusing time). Its strength is defined by the `gzlvl1` parameter and the diffusion delay by the `del` parameter. The actual DOSY setup determines the proper relationship among these three parameters. The best setting primarily depends on the sample itself (e.g., solvent, viscosity, molecular size and shape, the isotope to be detected) and on the experimental conditions (e.g., temperature). Therefore, it is recommended that you use the DOSY sample to optimize the experimental parameters. For small or medium sized molecules, it might be useful to set `gt1=0.002` and `del=0.05` sec and to array the gradient strength:

   `gzlvl1=500,5000,15000,20000,25000,30000` for Performa II

   `gzlvl1=50,500,1000,1500,2000` for Performa I gradient systems
6. For the maximum gradient power used in the DOSY experiment, select the `gzlvl1` value, which attenuates the signal intensities to 5% to 15% of the intensities obtained

with the weakest gradient pulse. If the intensity drop is not sufficient at the end of the array, you can increase `del` or `gt1`. If no signal is detected towards the end of the array, decrease `del` or `gt1` and repeat the procedure again.

7. Before the final setup, optimize the alfa delay to reach ideal baseline performance.

8. After having determined suitable values for `gt1`, `del`, and the maximum gradient power, call the `setup_dosy` macro.

   `setup_dosy` asks for the number of gradient levels, for the weakest and strongest gradient power to be used in the experiment and sets up a range of `gzlvl1` values with their squares evenly spaced. The minimum gradient strength may be set to 0.3-0.5 G/cm.The number of different pulse areas to use depends on the range of diffusion coefficients to be covered and the balance between systematic and random errors but typically is in the range of 10 to 30. As in any quantitative experiment, there is a balance to be struck when choosing a repetition rate between signal-to-noise and accuracy. But in DOSY experiments, a delay of 1-2 T_1 suffices, provided that care is taken to establish a steady state before acquiring data. It is recommended to set `ss<0` to have steady-state pulses at every new array element and run the acquisition interleaved (`il='y'`).

Each sequence is equipped with a Tcl-Tk acquisition panel, which provides direct access to parameters and setup related commands. Figure 5 shows the acquisition panel of the Doneshot sequence.

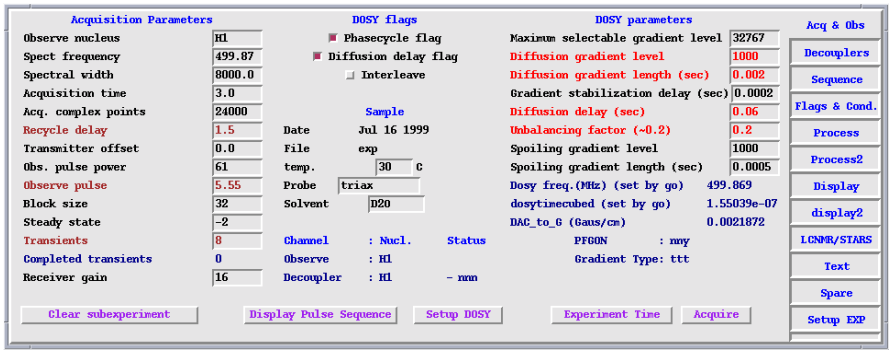


Figure 5. Tcl-Tk Acquisition Panel of Doneshot Pulse Sequence

*Dbppste (**DOSY** **Bipolar** **Pulse** **Pair** **St**imulated **E**cho Experiment)*

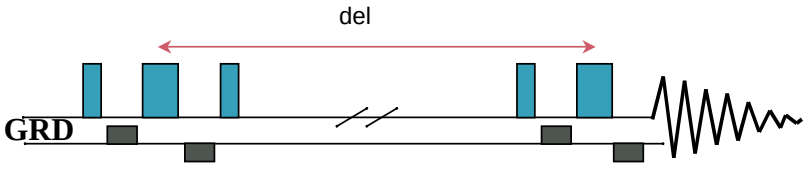


Figure 6. Dbppste Experiment

Table 8. Dbppste Parameters

Parameters	Function
`calibflag`	Correct systematic errors in DOSY experiments.
DAC_to_G	Store gradient calibration value in DOSY sequences.
`del`	Actual diffusion delay.
`delflag`	`y` runs the Dbppste sequence.
	`n` runs the normal s2pul sequence.
`fn2D`	Fourier number to build up the 2D display in F2.
`gstab`	Gradient stabilization delay (~200-300 us)fn2D.
`gt1`	Total diffusion-encoding pulse width.
`gzlvll`	Diffusion-encoding pulse strength.

*DgcsteSL (**D**OSY **G**radient **C**ompensated **St**imulated **E**cho with **S**pin **L**ock) Experiment*

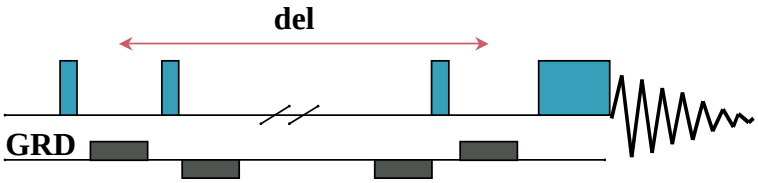


Figure 7. DgcsteSL Experiment

Table 9. DgcsteSL Parameters

Parameters	Function
`calibflag`	Correct systematic errors in DOSY experiments.
DAC_to_G	Store gradient calibration value in DOSY sequences.
`del`	Actual diffusion delay.
`delflag`	`y` runs the DgcsteSL sequence.
	`n` runs the normal s2pul sequence.
`fn2D`	Fourier number to build up the 2D display in F2.
`gstab`	Gradient stabilization delay (~200-300 us).
`gt1`	Total diffusion-encoding pulse width.
`gz_alt`	Flag to invert the gradient sign on alternating scans (default is `n`).
`gzlvl1`	Diffusion-encoding pulse strength.
`prg_flg*`	`y` selects purging trim pulse (default).
	`n` omits purging pulse.
`prgpwr*`	Power level for the purge pulse.
`prgtime*`	Purging pulse length (in second).
`tweek`	Tuning factor to limit eddy currents. It can be set between 0 and 1, usually set to 0.0.

* The optional purging pulse can effectively eliminate the dispersion signal components. Be careful not to create convection in the sample by the trim pulse.

The “Oneshot” Experiment

The total gradient power transmitted to the sample remains independent on the phase encoding gradient power. Although the sequence design makes phase cycling unnecessary and, unlike other DOSY sequences, the Doneshot sequence can be run with a single transient per array element, it is recommended to turn on the cyclops cycle:
`phasecycleflag='y'`

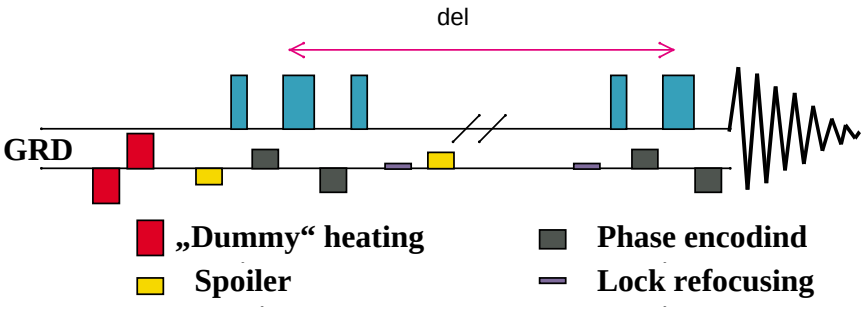


Figure 8. Oneshot DOSY Experiment

The lock refocusing gradient is determined by `kappa` and `gzlvl1`. The dummy heating gradients are automatically adjusted by the sequence. For the maximum gradient power available in the experiment, use:
`gzlvl_max > gzlvl1*(1+kappa)`

Table 10. Oneshot DOSY Parameters

Parameters	Function
`calibflag`	Correct systematic errors in DOSY experiments.
DAC_to_G	Store gradient calibration value in DOSY sequences.
`del`	Actual diffusion delay.
`delflag`	y runs the Doneshot sequence.
	n runs the normal s2pul sequence.
`fn2D`	Fourier number to build up the 2D display in F2.
`gstab`	Gradient stabilization delay (~200-300 µs).
`gt1`	Total diffusion-encoding pulse width.
`gt3`	Spoiling gradient duration (sec).
`gzlvl1`	Diffusion-encoding pulse strength.
`gzlvl3`	Spoiling gradient strength.
`gzlvl_max`	Maximum gradient strength accepted.
	(32767 with Performa II or III, 2047 with Performa I)
`kappa`	Unbalancing factor between bipolar pulses as a proportion of gradient strength (recommended:~0.2).
`phasecycleflag`	Flag to turn on or off the phase cycle.

*Dbppsteinept (**D**OSY **B**ipolar **P**ulse **P**air **St**imulated **E**cho **INEPT**) Experiment*

This sequence uses the higher “resolving power” of the wide ^{13}C chemical shift range, while the phase encoding and decoding step is more effectively done on the ^{1}H magnetization.

Table 11. Dbppsteinept Parameters

Parameters	Function
`calibflag`	Correct systematic errors in DOSY experiments.
DAC_to_G	Store gradient calibration value in DOSY sequences.
`del`	Actual diffusion delay.
`delflag`	`y` runs the Dbppsteinept sequence. `n` runs the normal INEPT sequence.
`fn2D`	Fourier number to build up the 2D display in F2.
`gt0`	Gradient duration of the spoiler gradient (for `satflag='y'`).
`gt1`	Total diffusion-encoding pulse width.
`gzlvl0`	Spoiling gradient power.
`gzlvl1`	Diffusion-encoding pulse strength.
`j1xh`	One-bond H-X coupling.
`mult`	Carbon multiplicity. 2 selects CHs (doublets). 3 gives CH2s down and CHs CH3s up. 4 enhances all protonated carbons.
`pp`	90° hard ^{1}H pulse.
`pplvl`	Decoupler power level for pp pulses.
`satflag`	`y` gives a grad-90(X) grad sequence during the diffusion delay to destroy the X-magnetization not originated from INEPT transfer.

Processing 2D-DOSY Experiments

After DOSY data has been acquired, it must be processed to give a 2D DOSY spectrum. Processing data involves the following stages:

1. Reference deconvolution with the command `fiddle` (optional, but useful if there is a suitable reference line that slowly diffuses).
2. Baseline correction with the macro `fbc` (also optional, but strongly recommended).
3. Extraction of diffusion data from the spectra and synthesis of a 2D DOSY spectrum with the macro `dosy`.

fiddle

The `fiddle` program enables you to use reference deconvolution to correct the line shapes, frequencies, phases, etc. of the signals caused by instrumental imperfections. Full instructions for its use are given in section 6.2 “Deconvolution” on page 207 and in the *Command and Parameter Reference* manual. Reference deconvolution of DOSY spectra removes systematic errors caused by the disturbance of the magnetic field and field/frequency lock caused by gradient pulses. It is best to use `fiddle` with the `writefid` option to store the corrected data, recall the corrected data, and set all the weighting parameters to `n` before Fourier transforming and proceeding to the next step.

fbc

The `fbc` macro applies `bc` type baseline correction to all spectra in an array. Use the partial integral mode to set integral regions to include all significant signals. Leave blank as large an area of baseline as you possible can; this minimizes systematic errors in diffusion coefficient fits caused by baseline errors.

dosy

This macro uses the commands `dll` and `fp` to determine the heights of all signals above the threshold defined by the parameter `th`. Then it fits the decay curve for each signal to a Gaussian using the program `dosyfit`, storing a summary of all diffusion coefficients and their estimated standard errors and various other results as follows:

In the directory `$HOME/vnmrsys/Dosy`:

- diffusion_display.inp
- general_dosy_stats
- calibrated_gradients
- fit_errors
- diffusion_spectrum

In the current experiment:

- A second copy of diffusion_display.inp.

The `dll` program is limited to handling 512 lines, so very crowded spectra might need to be processed in sections by appropriately choosing `sp` and `wp`. `dosy` then runs the command `ddif` to synthesize the 2D DOSY spectrum.

The peak representation and the accuracy of the peak heights might increase with higher digital resolution, i.e., zero-filling the FIDs (`fn>np`) can occasionally improve the quality of the DOSY data. In extreme cases, even `fn=512k` is allowed by the software. Building up a 2D data set (and a 2D display) with this data size would not make sense; therefore a new parameter, `fn2D` (with a maximum limit of 64k), is introduced in the 2D-DOSY sequences, replacing `fn` when setting up the 2D display.

Note: The 2D DOSY display is set up in the same experiment where the data processing takes place.

The synthesized spectrum contains `fn1/2` traces in the diffusion domain (`f1`), and `fn2D` real data points in the spectral domain (`f2`); `fn1` is limited to the range 128-1024. Normally `fn2D` of 16k suffices. If `fn2D*fn1` is too large, spectral synthesis and display will be slow and/or VNMR might run out of disk space.

Note: After displaying a 2D spectrum, the variable `ni` is set to `fn1/2` (this setting is required by `dconi`). So if more data is to be acquired or the sequence is to be displayed (`dps`) you must set `ni` back to zero.

By default, `dosy` uses all the experimental spectra and covers the whole diffusion range seen in the experimental peaks. Either one or three arguments, shown in Table 12, can be supplied to `dosy` to change the defaults.

Table 12. `dosy` Commands

Command	Function
`dosy('prune')`	Start a dialog to allow one or more spectra to be omitted from the analysis.
`dosy(d1,d2)`	`d1` and `d2` are numbers causing the diffusion range of the synthesized spectrum to be limited to $d1*10^{-10}$ m^2/sec and $d2*10^{-10}$ m^2/sec.
`dosy('prune',d1,d2)`	Combine the previously described arguments.

The message `Systematic Gz deviations` indicates that the random errors in the data are sufficiently small. It might be worthwhile correcting for the small systematic errors in the field gradients, produced by the spectrometer hardware, by using the decay curves of selected signals to provide an internal calibration of the relative gradient strengths. To correct for systematic gradient errors, do the following procedure:

1. Set the display/threshold parameters to select a few strong, well-resolved signals, which are known to arise from single species (i.e., the signals are not composites of overlapping signals from species with different diffusion coefficients). Enter `dosy` to perform the analysis a first time, readjust the display and threshold to contain all the signals of interest.
2. Enter `undosy calibflag='y' dosy`. The second analysis uses the shapes of the decay curves in the first analysis to correct for systematic errors. Remember to set `calibflag` back to `'n'` if you wish to stop using the internal gradient calibration.

WARNING: **If the argument `prune` was used for the initial run of the `dosy` macro, you must ensure that the same increments are deleted in the second run. Use `undosy calibflag='y' dosy('prune')` and specify the same increment number(s).**

The two-dimensional DOSY display (and plot) is constructed by taking the bandshape of a given signal from the first (lowest gradient area) spectrum and convoluting it in a second dimension with a Gaussian line centred at the calculated diffusion coefficient and with a width determined by the estimated error of the diffusion coefficient obtained from the fitting process.

To extract spectra of the mixture components separated along the diffusion axis, select the region of interest using the two cursors in the interactive 2D display (`dconi`) mode and click on `Proj` (projection) and `Hproj(sum)` (horizontal projection). The spectrum can be plotted with the `Plot` menu.

When processing 2D DOSY spectra, you might find the Tcl-Tk process panel, shown in Figure 9, useful..

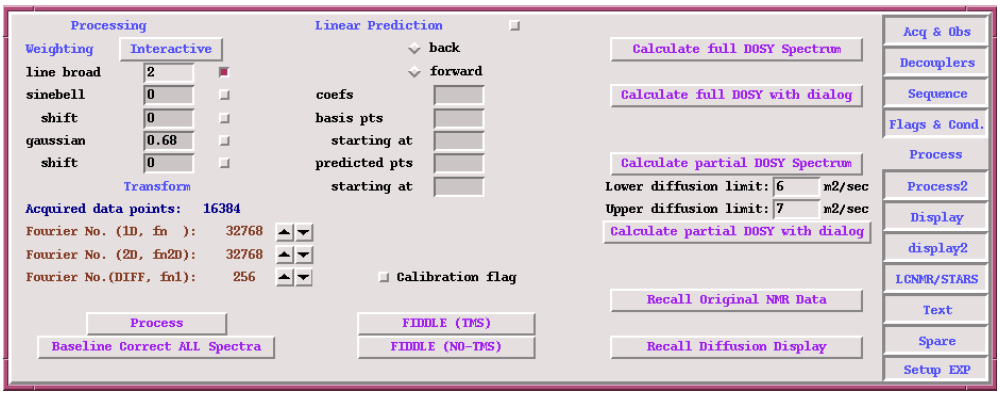


Figure 9. Tcl-Tk Process Panel for 2D_DOSY Pulse Sequences

WARNING: **Do not process the data with the `dosy` macro until the acquisition has been completed.**

sdp

The `sdp` macro (show diffusion projection) displays the integral projection of a DOSY dataset onto the diffusion axis. `sdp` uses the file `userdir+'/Dosy/diffusion_spectrum'` as input for the `sdp` command. Only use `sdp` in an experiment in which data can be overwritten because it modifies parameters such as `sw` and `at`.

3D-DOSY Experiments

3D DOSY adds a diffusion domain to "conventional" 2D experiments such as COSY or HMQC. The package contains sequences for DOSY-COSY (Dgcstecosy) and DOSY-HMQC (Dgcstehmqc), but it is easy to add diffusion encoding to many other 2D experiments. The 3D DOSY sequences provide better resolving power than the 2D counterparts (the probability of overlapping cross-peaks in 2D is much lower than the probability of overlapping lines in 1D proton detected experiments), at the expense of data size and experiment time.

An arrayed set of 2D experiments is performed using different values of gradient strength (`gzlvl1`). The data is doubly Fourier transformed, and the first 2D spectrum is used to manually define 2D integral regions. `dosy` analyzes then fits the integral volumes in successive increments to Gaussians and synthesizes 2D integral projections of the 3D dataset between defined diffusion limits. Full 3D display is not implemented; although with patience, you can achieve a similar effect by performing a series of projections.

Setting Up 3D-DOSY Experiments

1. Make sure that the "conventional" parameters of the COSY / HMQC experiment, such as pulse widths, transmitter offset, spectral window are correctly set.
2. As with 2D DOSY, try to find suitable lower and upper bounds for the gradient strength `gzlvl1`. There is no need to run 2D experiments for this purpose; the first increment from a 2D run is normally adequate (`ni=1`).
3. In a COSY experiment with higher quantum filter (`qlvl`>1), the first increment does not contain signals. Set the incremented delay (`d2`) to 0.05-0.1 during the gradient optimization process. Set `d2` back to zero when starting the DOSY-COSY experiment.
4. Use the `setup_dosy` macro to set up an array of 5 to 10 different `gzlvl1` values.

The full 3D experiments then can be acquired. Note the total experiment time when choosing the number of `gzlvl1` values, `ni` and `nt`. Both sequences are equipped with a Tcl-Tk acquisition panel, shown in Figure 10.

Figure 10. Tcl-Tk Acquisition Panel for the Dgcstecosy Pulse Sequence

*Dgcstecosy (**D**OSY **G**radient **C**ompensated **S**timulated **E**cho **COSY**) Experiment (AV mode)*

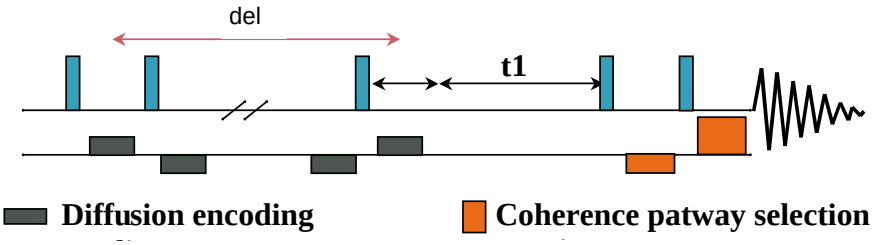


Figure 11. Dgcstecosy (AV Mode) Experiment

Using the `('t2dc')` argument to `wft2d` can be useful.

Table 13. Dgcstecosy Parameters

Parameters	Function
`calibflag`	Correct systematic errors in DOSY experiments.
DAC_to_G	Store gradient calibration value in DOSY sequences.
`del`	Actual diffusion delay.
`gt1`	Total diffusion-encoding pulse width.
`gzlvl1`	Diffusion-encoding pulse strength.
`gstab`	Gradient stabilization delay (~200-300 us).
`tweek`	Tuning factor to limit eddy currents. (can be set between 0 and 1, usually set to 0.0).
`gt2`	Gradient duration for pathway selection.
`gzlvl2`	Gradient power for pathway selection.
`qlvl`	Quantum filter level (1= single quantum, 2=double quantum).

*Dgcstehmqc (**D**OSY **G**radient **C**ompensated **S**timulated **E**cho **HMQC**) Experiment (AV mode)*

Process N-type data with `wft2d(1)` to process the first 2D experiment.

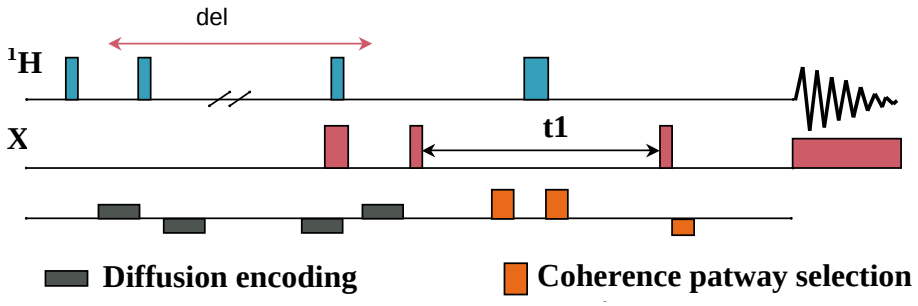


Figure 12. Dgcstehmqc Experiment (AV Mode)

Table 14. Dgcstehmqc Parameters

Parameters	Function
`del`	Actual diffusion delay.
`gt1`	Total diffusion-encoding pulse width.
`gzlvl1`	Diffusion-encoding pulse strength.
`gt2`	First coherence pathway selection gradient in HMQC.
`gzlvl2`	Gradient power for gt2.
`gt3`	Second coherence pathway selection gradient in HMQC.
`gzlvl3`	Gradient power for gt3.
`gt4`	Refocusing gradient in HMQC.
`gzlvl4`	Gradient power for gt4.
`gstab`	Gradient stabilization delay (~200-300 us).
`pwx`	90° X pulse.
`pwxlvl`	Power level for `pwx`.
`j`	One-bond H-X coupling.
`mbond`	Flag to select multiple-bond correlation (HMBC).
`taumb`	Delay for magnetization transfer for `mbond='y'`.
`c180`	Flag to use the 180° X pulse a composite pulse.

If `gzlvl4` has the same sign as `gzlvl2` and `gzlvl3` (N-type selection), use `wft2d` to process the data.

If `gzlvl4` has opposite sign to `gzlvl2` and `gzlvl3` (P-type selection), use `wft2d('ptype',1)` to process the data. Using the (`'t2dc'`) argument to `wft2d` can be useful.

Before using the sequence for the first time the coherence pathway selected gradients needs to be calibrated for a given probe and gradient amplifier.

The choice of decoupling method in the DOSY-HMQC experiment is crucial, as even relatively low values of `dpwr` can cause sufficient convection currents to invalidate DOSY results. On UNITY*plus* or UNITY*INOVA* systems equipped with a PPM module in the ^{13}C channel adiabatic decoupling schemes (WURST, STUD) is recommended.

Processing 3D-DOSY Experiments

In order to analyze 3D results, it is necessary to manually define the individual signal regions in the 2D spectrum.

1. 2D Fourier transform the first increment of the 3D data set (i.e., the increment with the lowest `gzlvl1` value), using proper weighting functions in both dimensions:
   ```
   wft2d(1) for COSY
   wft2d('ptype',1) for HMQC
   ```
2. Correctly set `vs2d` and `th`, then define the signal regions in the first spectrum using the standard `ll2d` command and its options (e.g., `'reset'`, `'volume'`, `'clear'`, `'combine'`) The options are easily accessible via the `dconi/Peak/Edit` menu set.
3. Include all the components of a given multiplet (cross-peak) in a single integral region, provided that there is no contamination by other signals. Grouping signals in this way maximizes the signal-to-noise ratio available for data fitting.

   This step offers you the unique opportunity to exclude apparent spectral artifacts (t1-noise, decoupling sidebands, spurious peaks, etc. from the DOSY analysis.) Because the manual peak selection is probably the most boring and time-consuming step of the whole procedure, after it is completed, it is worth storing the file (using the command `ll2dbackup`) in the same directory where the FID is stored for later processing.
4. After the signal regions have been defined, enter the command `dosy`.

   The macro extracts the volume of each region for every value of `gzlvl1` (this step involves, among other things, as many 2D Fourier transforms as there are `gzlvl1` increments). `dosy` then fits the volumes as functions of `gzlvl1`, returning with a display in which each signal region is labelled with its diffusion coefficient (10^{-10} m^2/sec) and with its standard error in brackets. The coefficients are automatically displayed when the `dosy` macro is completed using the `label` facility of the `ll2d` command. Thus, 6.05(0.05) means a diffusion coefficient of $6.05*10^{-10}$ m^2/sec (+/- $0.05*10^{-10}$ m^2/sec). The 2D spectrum on which the display is based is that of the first 2D increment of the 3D experiment. A copy of the diffusion results is available from the file `userdir+/Dosy/diffusion_display_3D.inp`. This file contains three columns:
   - The peak number (as obtained by `ll2dmode='nynn'`)
   - The diffusion coefficient
   - The standard error
5. The display of diffusion coefficients as numbers on the screen can result in very crowded display. You can change the type of information shown by using the `ll2dmode` parameter (for details see the *Command and Parameter Reference Manual* and Figure 13).
6. In order to make the analysis easier, use `sdp` to obtain the integral projection of the 3D data set onto the diffusion axis. You can use this diffusion spectrum to choose suitable diffusion regions for which to examine 2D projections of the 3D DOSY data.

WARNING: **Warning: Be sure to use `sdp` in an experiment in which data can be overwritten!**

7. In the experiment containing the 3D data, enter the command:

```
makeslice(d1,d2)
```

where `d1` and `d2` are the diffusion limits (in units of 10^{-10} m^2/s) between which the 2D projection of the 3D DOSY spectrum is required. The `makeslice` macro builds the slice and displays it after a few seconds. `makeslice` uses, among other things, the diffusion information in the file `userdir+'/Dosy/diffusion_display_3D.inp'`.

8. To return to the original spectrum, enter `showoriginal`. This command reverts to the original 2D spectrum for the first value of `gzlvl1`.
9. You can repeat the sequence `makeslice` – `showoriginal` as needed with different diffusion values (or slice thicknesses), but you must use `showoriginal` in between the display of two slices.

   Both sequences are equipped with a Tcl-Tk Process2 panel, shown in Figure 13, providing access to all necessary functions and parameters to process 3D DOSY data.

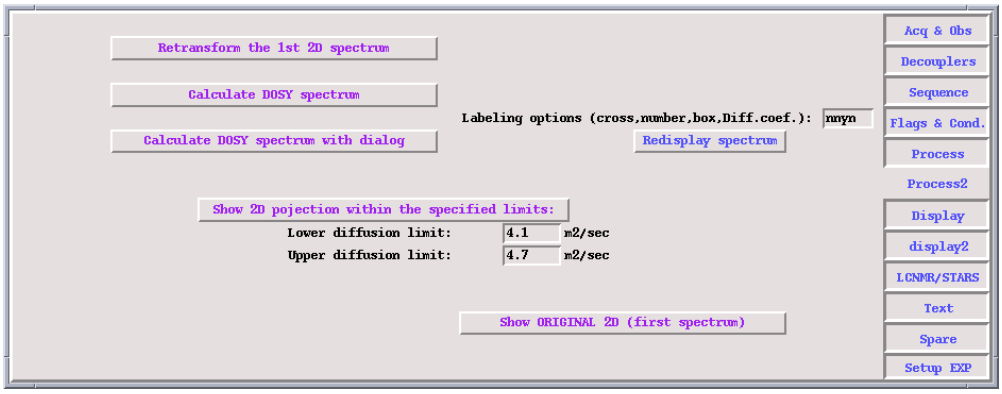


Figure 13. Tcl-Tk Process2 Panel for the 3D-DOSY Pulse Sequences

Sample FIDs to Practice DOSY Processing

The package includes a few 2D and 3D FIDs (in `/vnmr/fidlib/Dosy`) to practice DOSY processing. Except for Doneshot, every pulse sequence has an example. Processing Doneshot data is not unique; it requires the same procedure as the Dbppste or the DgcsteSL sequences.

Dbppste.fid

The sample is a mixture of three dipeptides (Phe-Val, Phe-Glu, and Phe-Gly) and 3 (trimethylsilyl)-1-propane-sulfonic acid dissolved in D_2O.

1. Load the data file into the experiment:
   `cd('/vnmr/fidlib/Dosy') rt('Dbppste.fid')`
2. Enter **`ft`** and adjust the phase of the first spectrum.
3. Set the cursor to the TSP singlet and enter **`nl rl(0)`**.
4. Change to a directory (`cd`) in which you have write permission.
5. Set the cursors 30 Hz either side of the TSP singlet, set **`lb='n'`** and **`gf=0.75`**, and enter the command
   `fiddle('satellites','TMS','writefid','temp')`

to perform reference deconvolution on all the data, regularizing the lineshapes so that the peak heights in successive spectra accurately reflect the signal integrals.

6. Recall the FID created in the previous step and retransform it:
   `rt('temp') gf='n' lb='n' ft`
7. The integral regions have already been set in the supplied parameters. Display the integral to see where the resets have been positioned.
8. Enter **`fbc`** to perform baseline correction.
9. Set the threshold below the peaks of interest:
   `(vs=500, th=3)`
10. Enter **`dosy`**.
11. To zoom into the diffusion region of interest, enter **`undosy dosy(4,7)`**.

The following examples describe how to process DOSY data in the command mode (Commands column) or by using the Tcl-Tk Process (2D-DOSY) or Process2 (3D-DOSY) panels (Buttons in the Process Window column). The middle column has instructions or comments about both types of operations.

DgcsteSL.fid

The sample is a mixture of adenosine mono-, di-, tri-phosphate (AMP, ADP, ATP) and K_2HPO_4 in D_2O (pH=7). The data was acquired in a 3mm probe with direct ^{31}P observe.

Commands	*Comments, Instructions for Both*	*Buttons in the Process Window*

	Recall the FID:
	`cd('/vnmr/fidlib/Dosy')`
	`rt('DgcsteSL.fid')`	

`lb=2 wft`	Fourier transform	**Process**
`fbc`	Do baseline correction	**Baseline correct All spectra**
`dosy`	Execute dosy.	**Calculate full DOSY spectrum**
	To have better diffusion resolution, calculate a partial dosy spectrum:	
`undosy`		**Recall original NMR data**
`dosy(6.1,7.1)`		**Calculate partial DOSY spectrum**
	To display (and plot) the diffusion spectrum, join another experiment and execute `sdp`.	

Dbppsteinept.fid

The sample is a mixture of sucrose, methyl-alfa-D-glucopyranosid, 1,3,5,-O-methylidene-mio-inosytol, and dioxane (as internal reference) in D_2O. The experiment was run using an AutoSwitchable gradient probe.

Command	*Comments, Instructions for Both*	*Buttons in the Process Window*

	Recall the FID:
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dbppsteinept.fid')`	

`ft`	Fourier transform	**Process** (`unset lb` and `gf`)

Command	*Comments, Instructions for Both*	*Buttons in the Process Window*
`fbc`	Do baseline correction	**Baseline correct All spectra**
`lb=-0.4 gf=0.7`	Set weighting functions. Expand the spectrum and put the two cursors around the most intense line dixonane ± 15 Hz.	(Activate `lb` and `gf`)
`fiddle`	Execute fiddle.	**FIDDLE (No TMS)**
	Display full spectrum and set threshold.	
`dosy`	Execute dosy.	**Calculate full DOSY spectrum**
	To have better diffusion resolution, calculate a partial dosy spectrum:	
`undosy`		**Recall original NMR data**
`dosy(2.0,5.0)`		**Calculate partial DOSY spectrum**
	To display (and plot) the diffusion spectrum, join another experiment and execute `sdp.`	

Dgcstecosy.fid

The sample is a mixture of sucrose, methyl-alfa-D-glucopyranosid, and 1,3,5,-O-methylidene-mio-inosytol in D_2O. The experiment was run using an AutoSwitchable gradient probe.

Command	*Comments, Instructions for Both*	*Buttons in the Process2 Window*

	Recall the FID:
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dgcstecosy.fid')`	

`wft2d(1)`	Fourier transform.	**Retransform the 1st 2D spectrum**

	Signal regions for this file have already been saved. Recall `112d` file:
	`112d 112d('read'`
	`'Dgcstecosy.112d')`	

`112dmode='nnyn'`	Check preset regions. Each cross peak of interest is boxed.	**Labelling options: nnyn**
`dconi`		**Redisplay spectrum**
`dosy`	Execute dosy.	**Calculate DOSY spectrum**

Command	*Comments, Instructions for Both*	*Buttons in the Process2 Window*
	When ready, COSY spectrum is displayed again with each cross peak labelled by its diffusion coefficient and its error. Join another experiment and display diffusion projection: `sdp` A set of signals appear: 4.1-4.8–1,3,5,-O-methylidene-mio-inosytol 3.6-3.9–methyl-alpha-D-glucopyranosid 2.8-3.1–sucrose Other three lines between 3.2 and 3.6 D (10^{-10} m^2/sec) are overlapping diagonal peaks. Rejoin DOSY experiment.	
`112dmode='nnnn'`	Reset peak labels.	**Labelling options: nnnn**
`dconi`		**Redisplay spectrum**
	To display inosytol spectrum:	
`makeslice(4.1,4.8)`		**Low Lim.: 4.1, Up. Lim: 4.8** **Show 2D projection within . . .**
	To display glucopyranosid projection, recall original 2D.	
`showoriginal`		**Show original 2D (first spectrum)**
`makeslice(3.6,3.9)`	Display glucopyranosid. To display the last projection, recall the original 2D.	**Low. Lim.: 3.6, Up. Lim: 3.9**
`showoriginal`		**Show original 2D (first spectrum)**
`makeslice(2.8,3.1)`	Display sucrose.	**Low Lim.: 2.8, Up. Lim: 3.1** **Show 2D projection within . . .**

Note: By accident, this cosy spectrum was run with an unusual parameter setting (`sw<>sw1`). The setting was absolutely unintended and should not affect the DOSY

processing. The operator (P.Sandor, Darmstadt) assures you that the sequence also gives proper results with adequate parametrization.

Dgcstehmqc.fid

The sample is a mixture of quinine, geraniol, and camphene (and TMS) in deutero-methanol. See *J. Magn. Reson.* (1998), **131:** 131-138.

Commands	*Comments, Instructions for Both*	*Buttons in the Process2 Window*

	Recall FID:
	
	`cd('/vnmr/fidlib/Dosy')`
	`rt('Dgcstehmqc.fid')`	

`wft2d('ptype',1)`	Fourier transform.	**Retransform the first 2D spectrum**

	Signal regions for this file have already been saved. Recall ll2d file:
	
	`ll2d`
	`ll2d('read','Dgcstehmqc.ll2d')`	

`ll2dmode='nnyn'`	Check preset signal regions. Each cross peak of interest is boxed	**`Labelling options: nnyn`**
`dconi`		**Redisplay spectrum**
`dosy`	Execute dosy.	**Calculate DOSY spectrum**
	When ready, HMQC spectrum is displayed again with each cross peak labelled by its diffusion coefficient and its error. Join another experiment and display diffusion projection: `sdp` A set of signals appears: 7.0-8.5 – quinine 10.0-11.6 – geraniol 14.0-15.4 – camphene Other lines around 18 D (10^{-10} m^2/sec) are methanol and TMS. Rejoin DOSY experiment.	
`ll2dmode='nnnn'`		**Labelling options: nnnn**
`dconi`		**Redisplay spectrum**
	To display quinine spectrum:	
`makeslice(7.0,8.5)`		**Low. Lim.: 7.0, Up. Lim: 8.5** **Show 2D projection within . . .**
	To display another projection, you need to recall the original 2D.	

Commands	*Comments, Instructions for Both*	*Buttons in the Process2 Window*
`showoriginal`		**Show original 2D (first spectrum)**
`makeslice(10.0,11.6)`	Display geraniol.	**Low. Lim.: 10.0, Up. Lim: 11.6**
		Show 2D projection within . . .
	To display last projection, recall original 2D.	
`showoriginal`		**Show original 2D (first spectrum)**
`makeslice(14.0,15.4)`	Display camphene.	**Low. Lim.: 14.0, Up. Lim: 15.4**
		Show 2D projection within …

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Johnson C.S. Jr., “Diffusion-Ordered Nuclear Magnetic Resonance Spectroscopy: Principles and Applications,” *Progress in Nuclear Magnetic Resonance Spectroscopy* (1999), **34:** 203-256.

Filter Diagonalization Method

Filter Diagonalization Method (FDM) is a non-Fourier data processing method that extracts spectral parameters (peak positions, line widths, amplitudes, and phases) of Lorentzian lines directly from the time-domain signal by fitting FID data to a sum of damped complex sinusoids. The spectral parameters (saved in `curexp/datdir/fdm1.parm`) are also called “line list” and are used to construct an “ersatz” spectrum of the NMR data.

FDM is slower than Fast Fourier Transform, but it offers better resolution in the case of truncated signals and the option of processing only a selected spectrum region. FDM has the potential to work well with corrupted data, and the potential to produce a line list with each line represents a true NMR peak.

FDM reads input parameters from a file created by the `fdm1` macro, using default (optimal) values. You can change any of the parameters from the command line. Table 15 lists `fdm1` parameters. If the spectrum is not referenced with `rl`, the reference `rfl` is also read from `curpar`. The section “Changing Local Variables,” page 70 describes how you can override the default setting.

In most cases, you only need to decide the number of data points to be used and the spectrum window to be processed. By default, half of the FID data or 3000 data points, whichever is smaller, is used.

The window to be processed is determined by VNMR parameters `sp` and `wp`. If the data is already processed (using FT or FDM) and displayed, you can process the displayed region again with FDM by typing `fdm1`. If the data is not processed and displayed, `sp` and `wp` are read from the `curpar` file, but `sp` and `wp` might not be what you want.

Using FDM

The following steps describe how to do normal activities such as phasing, zooming in, zooming out, and processing a spectrum window with the `fdm1` macro.

1. Display the FID data and use the right mouse button to select the data points to be used by FDM.
2. Process the data with `ft` (it uses all FID points), then display and reference the spectrum.
3. Place the cursor on a region of interest, zoom in on it, then type `fdm1` or select the **Process2** panel and click on **1D FDM** to process the data. If you select but do not zoom in on a region, the whole spectrum in display is processed.

   A new menu appears with **Stop FDM** and **Display** buttons. The calculation might take a few seconds to a few minutes depending on the number of data points used and the size of spectrum window to be processed. To abort the process, click on **Stop FDM**. To check if the process is finished, click on **Display**. If the process is finished, display the spectrum.

Reprocessing a Spectrum

The **1D FDM** button is displayed on the **Process2** panel. Use this button to reprocess a spectrum.

Changing Parameters

Relevant `fdm1` global parameters are displayed on the **Process2** panel with current values. You can change these parameters. The value of a global parameter is saved to `curpar` and it remains the same until you change it from the parameter panel or make a new assignment using the command line. You can also change the parameters from the `fdm1` command line as described in the section "Changing Local Variables," page 70.

Global Parameters

The following FDM parameters are global.

Table 15. fdm1 Parameters

Parameter	Description
`cheat`	No cheat if `cheat=1`, lines are narrower if `cheat<1`.
`cheatmore`	No cheatmore if `cheatmore=0`.
`error`	Error threshold for throwing away poles.
`fdm`	1 for `fdm`, –1 for `dft`.
`Gamm*`	Smoothing width (line broadening).
`Gcut`	Maximum width for a pole.
`idat`	–4 for ASCII complex FID file, –5 for VNMR FID file.
`kcoef`	`kcoef>0`; use "complicated" `dk(k)`. –1 always preferred.
`Nb*`	Number of basis function in a single window.
`Nbc*`	Number of coarse basis vectors.
`Nsig*`	Number of points to use, 3000 is ok.
`Nskip*`	Number of points to skip.
`rho`	`rho=1` is optimal.
`ssw`	A test parameter.
`t0`	Delay of the first point.
`theta`	Overall phase of FID (`rp` in radians).
`wmin`	Minimum spectrum frequency in hertz.
`wmax`	Maximum spectrum frequency in hertz.

* Global; see "Global Parameters," page 69 for more information.

- `Nsig` is the number of FID points to use. You initialize it with the right mouse button position (`crf` + `deltaf`) `*sw`. If `Nsig=0`, half of the FID data points or 3000, whichever is smaller, is used. `Nsig` can be changed from the parameter panel, the command line `Nsig=nnnn`, the right mouse button (when the FID is displayed), or the command line `fdm1('Nsig',nnnn)`. In general, the more peaks you have, the more data points it takes to fit the spectrum. To check the reliability of the FDM method, change `Nsig` a few times and reprocess the data to see if you get the same result.
- `Nskip` is the number of data points to skip at the beginning of a FID. By default, zero points are skipped. In some cases, you can improve baseline by skipping the first one or two points.
- `Nb` is the number of basis functions (poles) used to fit each of the windows in an FDM calculation. The default is 10. FDM breaks down the specified window into smaller windows. In general, bigger `Nb` gives better results, especially better baseline. Sensible values for `Nb` are between 10 and 50.
- `Nbc` is the number of additional poles (coarse basis functions) to be used. The default is zero, but setting `Nbc` to an integer larger than zero (typically 4-10) might improve the baseline.
- `Gamm` is the smoothing width (line broadening). The default is `0.2*sw/Nsig`, which is about a tenth of the FT resolution. Typical values are 0.1 to 1.0.

Using bigger `Nsig`, `Nb`, `Nbc`, or a spectral window significantly slows down the calculation.

Changing Local Variables

FMD parameters that are not commonly used are set as `fdm1` local variables. These parameters are listed with global parameters in Table 15. You can change local variables only from the `fdm1` command line. Parameter values are lost after the completion of the macro. To use a value again, you must reenter it; otherwise, `fdm1` sets the value to the default. To change more than one local variable, enter the variables from the same command line.

You can change any of the FDM parameters from the `fdm1` command line and you can change both global and local variables. Values entered from the `fdm1` command line override the default, the change from the **Process2** panel, and the value that you select with the cursor. Enter command line arguments by giving the parameter name in single quotation marks and a value separated by a comma, for example:

```
fdm1('cheat',0.8)
fdm1('Nsig',3000)
fdm1('Nsig',3050)
fdm1('Nb',20)
fdm1('Nbc',10,'Nb',20)
fdm1('Nsig',3000,'Nb',20,'Gamm',0.5)
fdm1('wmin',-1600,'wmax',1600)
fdm1('wmin',-1600,'wmax',0)
```

`cheat` is a factor multiplied to the line width. There is no cheat when `cheat=1`; lines are narrower when `cheat<1`.

`wmin` is the minimum spectrum frequency in Hz. The default is `sp+rfl-sw/2`. `wmin` is the upper field.

`wmax` is the maximum spectrum frequency in Hz. The default is `wmin+wp`. `wmax` is the lower field.

The center of the full spectrum is zero.

Seeing Parameter Values

Parameters are set to their default values. Normally, you do not need to change these parameters or you might change some of the global parameters. You cannot inquire values of local `fdm1` parameters in the same way that you inquire global VNMR parameters with `echo` or `?`. To see the values of all parameters used, look in the `fdm1.inparm` file created by the `fdm1` macro in the `datdir` directory of the current experiment. Figure 14 shows the format of the `fdm1.inparm` file; the number of spaces and tabs is arbitrary.

```
fid_filename            idat
t0              theta
fdm
par
fn_Sp1D     spectype   axis
wmin            wmax
Nsig
Nskip
rho             Nb
error
Npower          Gamm            Gcut
cheat           cheatmore
Nbc     kcoef
ssw   sw
fidmt   specfmt
i_fid
```

Figure 14. `fdm1.inparm` File

References

J. Chen and V. A. Mandelshtam, *J. Chem. Phys.* (2000) **112:** 4429-4437.

V. A. Mandelshtam, *J. Magn. Reson.* (2000) **144:** 343-356.

A. A. De Angelis, H. Hu, V. A. Mandelshtam and A.J. Shaka, *J. Magn. Reson.* (2000) **144:** 357-366.

Chapter 2. 1D Experiments

Sections in this chapter:

- 2.1 “APT—Attached Proton Test,” page 74
- 2.2 “BINOM—Binomial Water Suppression,” page 75
- 2.3 “CPMGT2—Carr-Purcell Meiboom-Gill T2 Measurement,” page 76
- 2.4 “CYCLENOE—Cycled NOE Difference Experiment,” page 77
- 2.5 “D2PUL—Standard Two-Pulse Using Decoupler as Transmitter,” page 78
- 2.6 “DEPT—Distortionless Enhancement by Polarization Transfer,” page 79
- 2.7 “INEPT—Insensitive Nuclei Enhanced by Polarization Transfer,” page 82
- 2.8 “JUMPRET—Jump-and-Return Water Suppression,” page 83
- 2.9 “NOEDIF—NOE Difference Experiment,” page 84
- 2.10 “PRESAT—1D Water Suppression,” page 87
- 2.11 “S2PUL—Standard Two-Pulse Sequence,” page 87
- 2.12 “S2PULR—Standard Two-Pulse in Reverse Configuration,” page 88

This chapter describes a number of common 1D pulse sequences for “everyday” use. Each pulse sequence has a macro, with the same name as the pulse sequence, to help you set up the experiment. Many macros are written with the assumption that you have done a “normal” 1D experiment on the sample first. For example, after you have obtained a carbon spectrum, you can type `apt` to set up an APT experiment.

Each macro retrieves an associated file with parameters such as `pw90`, `tpwr`, `dmf`, etc. from a central location like `/vnmr/probe` or `$vnmruser/probe`. Other specific parameters, such as `mult` for DEPT and `d2` for APT, come from your user’s `parlib` directory or, if the file is not found there, from the system directory `/vnmr/parlib`.

It is important that these parameters be correct. The first time the macro for an experiment is entered (by typing `apt`, for example), the system retrieves the default parameters and values.

To change any of these values (for example, the default `d2` time in `apt` is 7 ms and you want the default to be 3 ms), make the appropriate change in the displayed parameters, and then save the modified parameters either in your user’s `parlib` or in the system `/vnmr/parlib`. Notice that because files in the directory `/vnmr/parlib` are available to all users, only the system administrator `vnmr1` has permission to save the files in this directory.

To view complete listings of each pulse sequence, print out or look at the contents of the files in the directory `/vnmr/psglib` on your system disk. You can also enter `dps` to view a graphical representation.

2.1 APT—Attached Proton Test

The `apt<(solvent)>` macro converts a ^{13}C parameter set to an APT experiment, where `solvent` is the name of the solvent to be used. If `solvent` is not supplied, `solvent` either defaults to $CDCl_3$ or, if in automation mode, `solvent` is read from the `sampleinfo` file.

Use half as many transients as the normal carbon 13, assuring multiples of 4. This must be done before changing other parameters because as soon as any parameter is changed, the number of completed transients is zero `(ct=0)`. Figure 15 is a diagram.

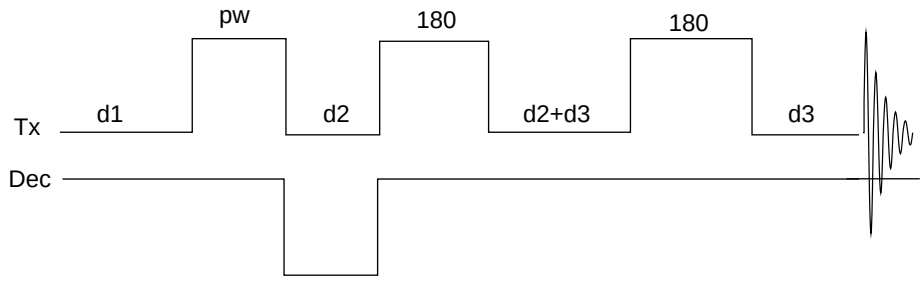


Figure 15. APT Pulse Sequence

Applicability

APT is available on all systems.

Parameters

`d2` is the `tau` delay (in sec): 3 ms nulls aromatic CH; 4 ms nulls all else except J > 125 Hz and quaternaries; 6 to 8 ms gives quaternaries, CH_2's up methyls; CH's down.

`p1` is a 180° pulse for observe nucleus (composite pulses used).

`pw` is a normal observe pulse. It need not be 90°.

`d3` is a short delay (typically 1 ms); if `d3=0`, the second 180° pulse is omitted and `pw` should be set to 90° or greater *(*`θ`*=180–*`θ`).

`dm='yny'` for an APT spectrum; `dm='yyy'` for a simple spin-echo spectrum.

`rof1` is set to 0 to turn receiver off during the entire pulse sequence.

`rof2` is the receiver dead time after last pulse in sequence.

Technique

To set up the experiment, enter `apt`. The `apt` macro sets up the experiment either by modifying an existing ^{13}C parameter set or by using default ^{13}C parameters. `apt` also sets the `τ` delay `d2` to 7 ms, which gives CH's, CH_3's down and CH_2's up.

References

Rabenstein; Nakashima, T. *Anal. Chem.* **1979**, *51*, 14651A.

Lecocq, C.; Lallemand, J. *J. Chem. Soc. Chem Comm.* **1981**, 150.

Patt, S.; Shoolery, J. *J. Magn. Reson.* **1982**, *46*, 535.

Related Commands and Macros

The `aptaph` macro automatically phases APT spectra.

2.2 BINOM—Binomial Water Suppression

The `binom` macro sets up parameters for the binomial water suppression sequence. Figure 16 is a diagram of the BINOM sequence with `seq=1510`.

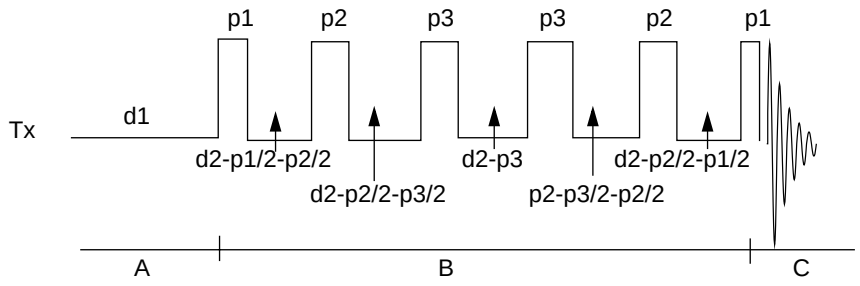


Figure 16. BINOM Pulse Sequence

Applicability

BINOM is not supplied on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`p1`, `p2`, `p3` are the pulses of the first half of the pulse sequence; all pulse sequences are symmetric (`p3` is used only for 1-5-10-10-5-1). If `p2=0` or `p3=0`, those pulses are derived from `p1`. If `p1=0`, `p1` is derived from `pw`, which is assumed to be a 90° pulse.

`offset` is the frequency, in Hz, from the carrier at which maximum excitation occurs with the binomial sequences. If `offset` > `0`, suppression is on-resonance. If `offset` < `0`, suppression is off-resonance (a null at the offset specified in Hz from the transmitter offset position) and `d2` is calculated by the program. If `offset=0`, a value of `d2` entered gives off-resonance suppression at the corresponding offset from the transmitter position.

`d2` is directly entered only if `offset=0`; otherwise, it is calculated.

`seq` is `11,121,1331,146` (gives 1-4-6-4-1), or `1510` (gives 1-5-10-10-5-1).

`rof2` is the receiver gating time, in µs, after last pulse in sequence.

`rof1` is the receiver gating time, in µs, before and after all other pulses. If `rof1=0`, the gate receiver is off during the entire sequence.

Reference

Hore, P. J. *J. Magn. Reson*. **1983**, *55*, 83–300.

Starcuk; Sklenar *J. Magn. Reson.* **1985**, *61*, 567–570.

2.3 CPMGT2—Carr-Purcell Meiboom-Gill T_2 Measurement

The `cpmgt2` macro modifies a parameter set to perform a Carr-Purcell Meiboom-Gill T_2 measurement. Figure 17 is a diagram of the CPMGT2 sequence.

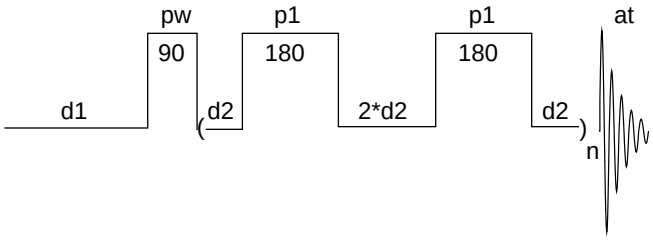


Figure 17. CPMGT2 Pulse Sequence

Applicability

CPMGT2 is available on all systems.

Parameters

`pw` is set for 90° pulse.

`p1` is set for 180° pulse.

`d1` is set for 1 to 3 times the T_1 value.

`d2` is the spin-echo cycle time; typical value is 1 to 10 ms.

`bt` ("big tau") is `4n` × `d2`, the total time for T_2 relaxation. Typically, `bt` is arrayed for a T_2 experiment. If `bt` is not a multiple of 4 × `d2`, it is rounded automatically so that it is.

T_2 Measurement

1. Run a spectrum of the material. Then enter the macro `cpmgt2`.

   This macro sets up the T_2 measurement parameters.

2. Create an array of `bt`. Values are entered in seconds. This array differs depending on the T_2's to be measured. An example is
   `bt=.020,.040,.080,.1,.120,.140`

   The computer determines the actual values used (selected as close as possible to the values you entered but still satisfying the equality `bt=4n` × `d2`). In the sequence diagram shown in <Red>Figure 12, the part in parentheses is repeated *n* times. The length of `bt` is limited, usually to about 0.5 seconds, but the upper limit varies depending on a variety of factors.

Acquisition and Processing

1. Enter `ga` to start data collection. After data collection completes, enter the commands below to process the data.
2. Enter `ds(1)` to display the first spectrum,
3. Click the mouse on the th button to obtain a threshold line, then place the threshold below the tops of the peaks of interest.

4. Enter `nll` to find the peak frequency.
5. Enter `fp` to find the top of the peak.
6. Enter `t2` to calculate and print T_2's of the peak.
7. If the exponential curve is not displayed, enter `expl`.
8. To plot the exponential curves, enter `pexpl`.
9. To plot the data, enter `dssh pl('all') pap page`.

2.4 CYCLENOE—Cycled NOE Difference Experiment

The `cyclenoe` macro sets up parameters for a cycled NOE difference experiment.

Applicability

CYCLENOE requires that the observe channel be equipped with direct synthesis rf and a linear amplifier. This experiment does not apply to *MERCURY-VX*, *MERCURY*, or *GEMINI 2000* systems; see the NOEDIF experiment instead.

Parameters

`pw` is a 90° excitation pulse (at power `tpwr`).

`intsub='y'` sets internal subtraction of data acquired by on-resonance and off-resonance selective excitation on alternate scans. `intsub='n'` makes data acquired by on-resonance and off-resonance selective excitation to be stored separately; only 1D with `satfrq` is collected.

`satfrq` is the frequency of selective saturation (on-resonance).

`control` is the off-resonance selective saturation frequency (an inactive parameter if `intsub='n'`). If `control` is inactive, off-resonance spectra is not collected.

`cycle='y'` does on-resonance saturation using frequency cycling around the frequency `satfrq` given by `spacing` and `pattern`; `cycle='n'` does off-resonance saturation at control.

`spacing` is the spacing, in Hz, of the multiplet.

`pattern` is the pattern type (1 for singlet, 2 for doublet, etc.). Fit the desired pattern to some value, even if some frequencies do not fall on NMR lines

`tau` is the time spent on a single irradiation point during cycling.

`satpwr` is the power of selective irradiation (typical values are at lowest power).

`sattime` is the total length of irradiation at frequency `satfrq`.

`mix` is the mixing time.

`sspul='y'` does `trim(x)–trim(y)` before `d1`.

`nt` is a multiple of 16 if `intsub='n'`, `nt` is a multiple of 32 if `intsub='y'`.

Technique

CYCLENOE does alternate scan subtraction of two FIDs in which the saturation frequency is moved on-resonance and off-resonance (for `intsub='n'`). Separate data tables are stored for `intsub='n'`. Power may be reduced from ordinary NOE experiments because the irradiation can be cycled (`cycle='y'`) through the lines of a multiplet.

1. One way to set up `satfrq` is by entering `dn='H1'` and using `sd` the same as in homodecoupling. Then enter `satfrq=dof` and `dn='C13'`.
2. Adjust proper `satpwr` by setting `nt=1` and arraying `satpwr` from 3 to –16 on UNITYINOVA and UNITY*plus* systems, and from 3 to 0 on UNITY and VXR-S systems. Enter proper values for `pattern` and `spacing`. `tau` is typically a few hundred milliseconds. `sattime` is usually several seconds.
3. Acquire the data and select the power necessary for 50 to 75% saturation. Then set `nt` to a large number (several hundred).
4. Set the control frequency as near as possible to `satfrq` to make the control and `satfrq` conditions as close as possible. The control frequency should be “in the noise,” not on top of a multiplet. Several protons may be done simultaneously for one control frequency.
5. Enter appropriate frequencies for patterns and spacings.
6. Set `array='(satfrq,pattern,spacing)'`. This performs the proper number of experiments.
7. Run nonspin and temperature-regulated. A large number of transients result in better subtraction.

2.5 D2PUL—Standard Two-Pulse Using Decoupler as Transmitter

The `d2pul` macro sets up parameters for a two-pulse sequence with the decoupler configured as a transmitter. Figure 18 is a diagram of the D2PUL sequence.

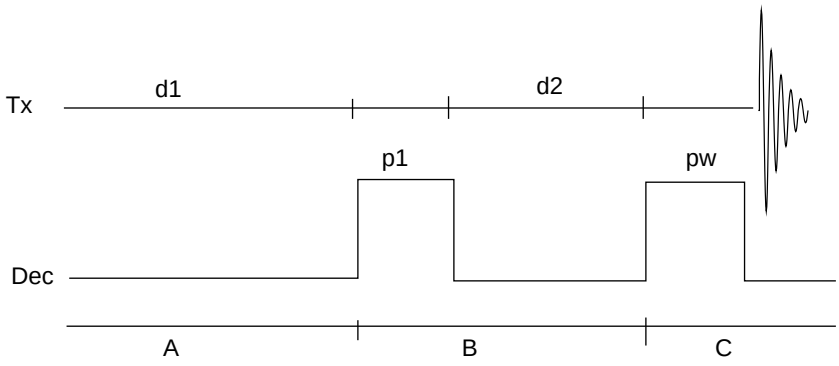


Figure 18. D2PUL Pulse Sequence

Applicability

D2PUL is available on all UNITY-series systems. On *MERCURY-VX* and *MERCURY* systems, the high-band transmitter is used as observe and decoupler—use S2PUL. On *MERCURY-VX*

and *MERCURY* and *GEMINI 2000* systems, if `tn` is `'H1'` and `dn` is not `'H1'`, the software now automatically uses the decoupler as the observe channel and the broadband channel as the decoupler channel. This channel swapping also makes the pulse sequence `d2pul` macro obsolete on the *GEMINI 2000*. If you want to run the equivalent of `d2pul`, set `tn='C13'` and `dn='H1'`, and then run `s2pul`.

Parameters

`dof` (decoupler offset) should equal `tof` (transmitter offset) for proper signal detection.

`homo` must be set to `'n'`.

`tpwr`, instead of `dpwr`, controls the decoupler power level during pulses on UNITY*INOVA*, UNITY*plus*, and UNITY systems equipped with a linear amplifier on the decoupler rf channel. `dpwr` controls the decoupler power level at all other times during a pulse sequence for this type of system configuration.

For systems with class C decoupler amplifiers, `dhp` is set to 255 by the pulse sequence statement `declvlon` during all pulses and is reset to its parameter value for all other times during the pulse sequence.

Technique

Acquire a normal spectrum, then enter `d2pul` to convert the parameter set.

2.6 DEPT—Distortionless Enhancement by Polarization Transfer

The `dept` macro sets up parameters for a DEPT pulse sequence, an improved version of the INEPT experiment. Figure 19 is a diagram of the DEPT sequence.

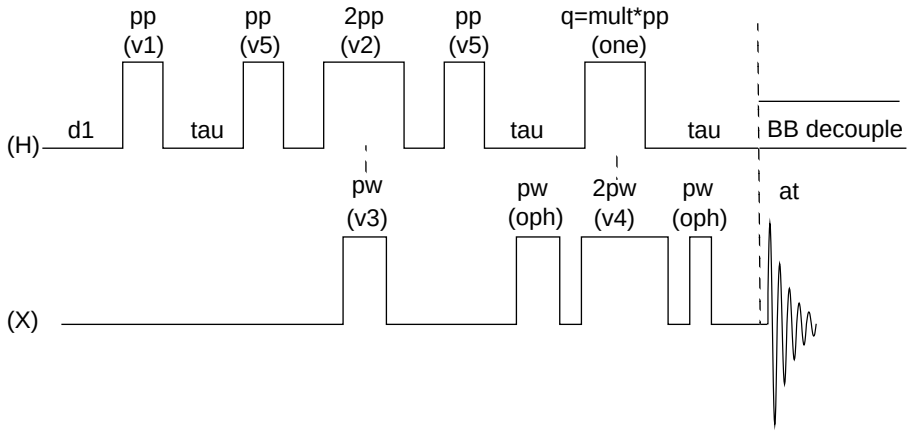


Figure 19. DEPT Pulse Sequence

Applicability

DEPT is available on all systems.

Parameters

`pw` is the 90° pulse (in µs) on the X-nucleus (usually ^{13}C) at power equal to `tpwr`.

`pp` is the proton 90° pulse width supplied from decoupler (refer to the decoupler 90° pulse width test using polarization transfer in the manual *Getting Started*). `pp` is set at the power level `pplvl` if linear amplifiers are present, or at full power if class C amplifiers are used. Optimum spectral editing requires a carefully calibrated value of `pp`. *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* broadband systems use linear amplifiers and the parameter `pplvl` must be set correctly. *GEMINI 2000* $^{1}H/^{13}C$ spectrometers do not have linear amplifiers and `pplvl` has no meaning.

`dmf` sets the modulation frequency for broadband decoupling of protons at power equal to `dhp` (if class C amplifier) or `dpwr` (if linear amplifiers). On *GEMINI 2000*, UNITY, and VXR-S systems, the modulation mode is WALTZ. On UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, and UNITY*plus* systems, a variety of modulation modes can be used. `dmf` sets the frequency for all modes.

`j` is an average X-H (usually C-H) coupling constant in Hz (`tau` is an internal parameter set to 1/2J).

`mult` is an arrayed parameter (0.5, 1, 1, 1.5) that leads to a value for the θ pulse of `mult*pp`: `mult=0.5` (θ = 45°) gives approximately equal excitation of all protonated carbons; `mult=1.0` (θ = 90°) excites CH's only (or mainly); `mult=1.5` (θ = 135°) gives CH's, CH_3's up, CH_2's down.

`d1` is a relaxation delay (1 to 3 times the value of t_1) for the protons connected to the NMR active nucleus, for example, ^{13}C or ^{15}N.

`at` is the acquisition time (t_2 period).

`nt` (number of transients) should be a multiple of 4. A multiple of 16 is suggested.

`dm` (decoupler modulation) is set to `'nny'`.

`satdly` is an optional saturation delay. If `satdly` is greater than 0, PW(90)-HS-SATDLY ^{13}C presaturation occurs immediately following the `d1` delay. If `satdly=0`, no PW(90)-HS-SATDLY ^{13}C presaturation occurs. The recommended value is `satdly=0.00`. (`satdly` is not available on *MERCURY-VX*, *MERCURY*, or *GEMINI 2000.)*

`hs='nn'` for no homospoil is recommended. If homospoil is used, `hst` (homospoil time) is typically 10 ms (`hs` must be `'nn'` on a *GEMINI 2000*).

Technique

To set up the experiment, enter `dept`. If a ^{13}C parameter set is present in the experiment, the `dept` macro modifies existing parameters; otherwise, it uses the default ^{13}C parameters. `dept` also sets `mult=0.5,1,1,1.5`.

Specific parameters for `dept` not found in the ^{13}C parameter set are retrieved from the user's `parlib` directory, or if not found there, from the `/vnmr/parlib` system directory. In either case, it is important that the parameters in these directories be correct.

To initiate acquisition, enter `au` if you wish automatic processing, spectral editing, and plotting. For auto processing, the parameter `wexp` should be set to `wexp='deptp'`. The edited spectrum is constructed as follows:

1. ALL: `0.23*(s1 + s2) + 2*s1`
2. CH: `s2 + s3`

3. CH2: `s1 – s4`
4. CH3: `–0.77*(s1 + s3) + s1 + s4`

where `s1` represents spectrum 1, `s2` represents spectrum 2, etc. Note that quaternary carbons are suppressed in the DEPT spectrum.

The `dept` experiment may be set up and run manually or by using the menu system. The following description uses the standard VNMR system menus and commands. Alternatively, the *GLIDE* system can be used to run the spectrum in a completely automated fashion.

1. A good sample to use the first time you run the experiment is 30% menthol in $CDCl_3$. Set up a carbon experiment by clicking on the **setup** menu button followed by the **C,CDCl3** button.
2. Set **`nt=4`** and enter **`dept`**. This sets up the experiment.
3. Enter **`ga`** to acquire the data. Four spectra are acquired, which you can edit.
4. After data acquisition, display the first spectrum by entering **`ds(1)`**.
5. Click on the **th** button to obtain a threshold line. Select a threshold level below the top of all peaks.
6. Enter **`adept dssa`** to analyze the spectra.
7. Enter **`pldept`** to plot the DEPT spectra.

Potential Problems

The most common failure of `dept` is poor subtraction in the edited spectra. Poor subtraction is usually caused by improper calibration of the decoupler 90° pulse `pp`. If the `dept` does not work, check the ^{13}C 90° pulse width, and decoupler 90° pulse width calibrations. Other causes of poor cancellation are lock saturation, leading to unstable lock, improper vibration isolation of the system, or temperature change during the experiment.

Reference

Doddrell, D.; Pegg, D.; Bendall, M. *J. Magn. Reson*. **1982**, *48*, 323.

Related Commands and Macros

The following macros assist in processing, analyzing, and plotting DEPT data:

- The `adept` command automatically analyzes a set of four DEPT spectra and edits the spectra so the spectra is arrayed in the following order: CH_3 carbons only, CH_2 carbons, CH carbons only, and all protonated carbons. `adept` produces a text file `dept.out` in the current experiment directory that contains the result of the analysis. Refer to the description of `adept` in the *VNMR Command and Parameter Reference* for the arguments available with `adept` and other information about the command.
- The `autodept` macro process DEPT spectra, plots the unedited spectra, edits the spectra, plots the edited spectra, and prints out editing information.
- The `deptgl` macro sets up parameters for a DEPTGL sequence (not distributed with *MERCURY-VX*, *MERCURY*, or *GEMINI 2000* systems) for spectral editing and polarization transfer experiments. Refer to the description of `deptgl` in the *VNMR Command and Parameter Reference* for the parameters used with `deptgl` and a reference to the literature.

- The `deptproc` macro weights, Fourier transforms, and phases each spectrum in a DEPT data set.
- The `padept` command performs the `adept` analysis and plots the resulting spectra with a scale and the assigned line listing. Refer to the description of `padept` in the *VNMR Command and Parameter Reference* for the keyword arguments used with `padept` and other information.
- The `pldept` macro plots out DEPT data, edited or not edited.

2.7 INEPT—Insensitive Nuclei Enhanced by Polarization Transfer

The `inept` macro sets up parameters for an INEPT pulse sequence. Figure 20 is a diagram of an INEPT sequence with `mult=3`.

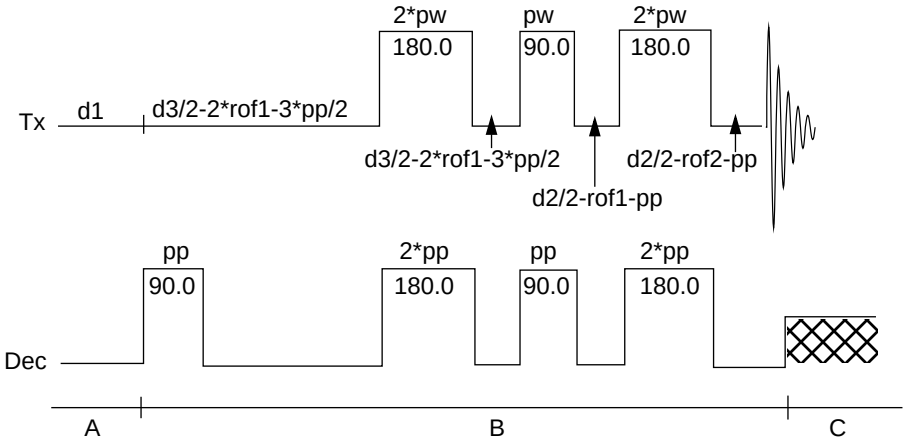


Figure 20. INEPT Pulse Sequence

Applicability

INEPT is available on all systems.

Parameters

`j` is a coupling constant (0 allows entry of `d2`, `d3`).

`pw` is a 90° pulse on the observe nucleus.

`pp` is a 90° pulse on protons using the decoupler.

`mult` selects multiplicity:
`mult=0` gives a normal experiment (`d1–pw–at` sequence);
`mult=2` selects ch's (doublets);
`mult=3` gives ch2's down, ch's and ch3's up; and
`mult=4` enhances all protonated carbons.

`dm='nny'` gives decoupled spectrum; `dm='nnn'` gives coupled spectrum.

`dmm` is set to `'ccw'` or `'ccf'`.

`focus='y'` gives refocusing for coupled spectrum (decoupled spectra are always refocused).

`normal='y'` gives normal multiplets in coupled spectra.

If the decoupler channel uses a linear amplifier, `pplvl` is the power level for the proton decoupler pulse and `dpwr` is the power level for broadband proton decoupling. If the decoupler channel uses a class C amplifier, maximum power is used for the proton decoupler pulse and `dhp` specifies the power level for broadband proton decoupling.

`d1` is a relaxation delay (1 to 3 times the value of t_1) for the protons connected to the NMR active nucleus, for example, ^{13}C or ^{15}N.

Technique

To set up the experiment, enter `inept`. If a parameter set is present in the experiment, the `inept` macro modifies existing parameters; otherwise, it uses the default parameters.

Specific parameters for `inept` not found in the spectral parameter set are retrieved from the user's `parlib` directory, or if not found there, from the `/vnmr/parlib` directory. In either case, it is important that the parameters in these directories be correct.

Reference

Morris, G.A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101*, 760.

2.8 JUMPRET—Jump-and-Return Water Suppression

The `jumpret` macro sets up parameters for the JUMPRET pulse sequence.

Applicability

JUMPRET is available on all systems except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*.

Parameters

`pw` is the 90° pulse width at power `tpwr`.

`p1` is a correction to second pulse width, typically 0 to 1 µs. Try increments of 0.025 µs.

`d2` is set for desired excitation maximum (typically 100 to 200 µs).

`lp` should be able to phase spectrum with small `lp` (use `calfa`).

`lfs, zfs`: try solvent suppression digital filtering:
for `wft('zfs')`, try `ssfilter=300`, `ssntaps=11`, and `ssorder=7`.
for `wft('lfs')`, try `ssfilter=100`, `ssntaps=200`, and `ssorder='n'`.

Reference

Plateau, P.; Gueron, M. *J. Amer. Chem. Soc.* **1982**, *104*, 7310.

2.9 NOEDIF—NOE Difference Experiment

The `noedif` macro converts a ^{1}H parameter set to perform the NOE (Nuclear Overhauser Enhancement) difference experiment. Figure 21 is a diagram of the sequence. The NOEDIF experiment performs subtraction directly in the computer memory, avoiding the necessity for an add–subtract step.

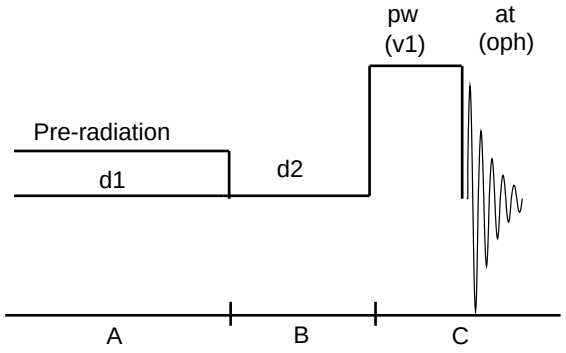


Figure 21. NOEDIF Pulse Sequence

Applicability

NOEDIF is available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* only. For UNITY-Series systems, see CYCLENOE.

Parameters

`d2` is set to 0 seconds.

`f0` to `f5` are individual decoupling frequencies through which to cycle (set unused frequencies to 0). `f6`, `f7`, `f8`, and `f9` are . . . can also be used.

You must start entering irradiation frequencies at `f0` through `f5`. If `f0` is left empty (0.0), then the entire irradiation period is skipped (as if `d1=0`).

`tau` is the time per individual decoupling (number of cycles equals `d1`/(`tau` × `n`), where `n` is the number of different frequencies).

`dofoff` is the position of decoupler during `d1` of control experiment.

`dof` is the position of decoupler during `d2` and acquisition.

`ctrl` is set to `'y'` for control experiment; set to `'n'` for decoupling cycling.

Phase Cycling

If `ctrl='y'`:

```
v1  =  +x  -x  +y  -y   +x  –x  +y  –y ...
oph =  +x  -x  +y  -y   +x  –x  +y  –y ...
```

If `ctrl='n'`:

```
v1  =  +x  +x  +y  +y   –x  –x  –y  –y ...
oph =  –x  +x  –y  +y   +x  –x  +y  –y ...
```

Procedure

The following procedure performs the NOE difference experiment on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* spectrometers. The order of the first three steps is critical.

1. Run a proton spectrum in the usual way, store the spectrum in `exp1`, then enter **`mf(1,2)`** to move the FID to `exp2`. Enter **`wft`**.
2. Enter **`noedif`**.

   The `noedif` macro converts the pulse sequence from S2PUL to NOEDIF and creates the parameters to set up the experiment. The screen displays instructions.
3. Enter **`ds`**. Find the peak or multiplet to be irradiated in the NOE difference experiment and expand the spectral region around it so that it occupies about 20 percent of the width of the screen. Display a cursor and select the frequencies for cycling during irradiation as follows:
   - If the line is a single line, multiple irradiation points may be chosen by setting the cursor at the point halfway up the line on the left side, then enter `sd` followed by `f0=dof`. Reset the cursor at the peak of the line, then enter `sd` followed by `f2=dof`. Next, set the cursor at the point halfway up the line on the right side, enter `sd` followed by `f3=dof`. The decoupler now cycles over these three frequencies, remaining at each frequency for a time equal to the parameter `tau` seconds. Enter `tau=0.1` unless it is already set to this value.
   - If the proton to be irradiated is a doublet, `f1` through `f4` are normally used, leaving `f5` at 0. Set up the frequencies `f1` to `f4` as described above, setting the cursor in sequence on the side of the first line, then on the second, then on the other side of the first line, and finally on the other side of the second.
   - Triplets and higher multiplets are usually cycled over the frequencies of each of the lines in the multiplet. Set the frequencies `f1` to `f#` (where # is the number of lines in the multiplet) as described above, putting the cursor on the peak of each line.
4. Enter **`ss=16`** to set the steady-state parameter `ss` to perform the sequence 16 times before acquiring data, allowing the magnetization of the nuclei to reach equilibrium state.
5. Enter **`d1=4`** to set the delay `d1` to 4 seconds. In samples with very long T_1 values, a longer delay is necessary to achieve equilibrium. Conversely, some samples with shorter T_1's may give good results with a shorter delay.

   The total number of steps equals `d1/tau`. Do not exceed 60 steps. If the number of steps exceeds 60, the acquisition parameters may be loaded into the acquisition computer by the host computer when the `go` command is given, but the acquisition computer may not be able to hold so many instructions and will not start to acquire data.

   If the values of `d1` and `tau` create more than 60 steps, increase the `tau` delay. For example, a `d1` of 10 seconds with a `tau=.1` produces 100 steps, which is too many. Increasing `tau` to 0.2 produces 50 steps, an acceptable number.

   An integral number of frequency cycles (`f1` through `f#`) is performed during `d1`. The computer changes `tau` if necessary to accomplish this.
6. Set **`dm='ynn'`** to turn on the decoupler during the delay period `d1`.
7. Set **`dofoff`** to determines where the decoupler is set to irradiate during alternate pulses when it does not irradiate the multiplet.

The default value of `doff` is –10000 Hz, but it can be set anywhere in the spectrum that is not close to a line, even within the spectral range of the observed lines. The exact position should not matter, but more than one setting may be tried to determine experimentally whether it does or not.

8. Set **`ctrl='n'`** to cycle the decoupler during the NOE difference experiment. Alternate FIDs are subtracted, beginning with the second pulse for which the decoupler is set to the `doff` position. This results in positive peaks for positive NOEs and a large negative signal for the irradiated peak or multiplet.

   If `ctrl='y'`, the decoupler is not cycled and alternate FIDs are not subtracted, so the resulting spectrum is the normal proton spectrum, obtained with the same pulse width and delay time as the NOE experiment. This gives the control spectrum to compare with the NOE difference spectrum. Both spectra can be obtained in one experiment by entering `ctrl='y','n'` to array the value of `ctrl`.

9. Set **`dpwr`** for *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* broadband systems, or **`dlp`** for $^{1}H/^{13}C$ *GEMINI 2000*, to give a value of decoupling field strength at the sample of about 2 to 3 Hz. This requires that the homonuclear decoupler is calibrated.

   - For *MERCURY-VX* and *MERCURY*, `dpwr` controls decoupler power in 1-dB steps, from 0 to 63.
   - For *GEMINI 2000* broadband, `dpwr` controls the homonuclear decoupler field strength, set in 0.5 dB steps from 0 to 50. If equipped with PIN-diode attenuators, additional fine control can be used through `dlp` on BB systems.
   - For *GEMINI 2000* $^{1}H/^{13}C$, `dlp` controls the proton homodecoupler power level (if present) from 0 to 2047 in arbitrary units, where 2047 is full power.

   The actual field strength can be calibrated exactly, but extreme accuracy is usually not necessary. Typical values vary widely from system to system:

   - On *MERCURY-VX* and *MERCURY*, use `dpwr` set to 10.
   - On *GEMINI 2000* broadband systems, start at `dpwr=5` and increase or decrease as necessary.
   - On *GEMINI 2000* $^{1}H/^{13}C$ systems, start at `dlp=1650` and increase in units of 50 until the correct power is obtained.

   A simple method to calibrate the decoupler for NOEDIF is to irradiate the peak of interest with a small amount of power and partially saturate the peak. Compare the spectra with and without irradiation (set `dm='ynn','nnn'`). When the peak intensity has decreased by 50 to 75%, the power is optimum for NOE difference.

10. Set **`bs='n'`** to disable block size storage.

11. Turn off the spinner and control sample temperature.

12. Run the NOE difference experiment for 512 pulses (if time permits). In general, the number of repetitions of the pulse sequence should be large enough to build up good signal-to-noise, since the NOE difference peaks are only a few percent of the normal spectral intensity, and should also be large enough to statistically average unavoidable perturbations of the field or frequency. A value of `nt` between 256 and 1024 should be suitable for most work unless very small samples are studied.

    Because the NOE difference experiment measures small differences between large signals, stability is very important. Be sure the spectrometer is protected from perturbing effects such as building vibrations, magnetic noise in the immediate vicinity (like moving iron objects and scanning magnetic fields), or strong air currents.

13. One possible cause of poor cancellation in this experiment is the noise in the lock channel. Set lock power to just below (3 dB) saturation and turn lock gain down as far as possible. The system remains locked at lock levels of 20%.

    The optimum combination of lock gain and lock power must be determined for each sample. A series of experiments with `nt` set to 16 or 32 should allow the best combination to be found without investing too much time. Then the value of `nt` should be increased and the data collected.

Reference

Kinns, M.; Sanders, J. K. M. *J. Magn. Reson.*, **1984,** *56*, 518.

2.10 PRESAT—1D Water Suppression

The `presat` macro sets up a standard two-pulse sequence with optional composite observe pulse: `{trim(x)trim(y)}..d1..satdly..p1..d2..pw..at`.

Applicability

PRESAT is available on all systems with a linear amplifier on the observe channel. It is not available on *GEMINI 2000* systems.

Parameters

`satmode` set to `'y'` gives observe transmitter saturation at `satfrq` with power `satpwr` (use like `dm`, e.g., `satmode='yyn'` or `satmode='ynn'`)

`sspul='y'` does `trim(x)trim(y)` to destroy all magnetization.

`composit='y'` uses composite 90° for `pw` (discriminates relative to B_1).

2.11 S2PUL—Standard Two-Pulse Sequence

The `s2pul` macro converts the current experiment into an experiment suitable for the S2PUL pulse sequence.

Applicability

S2PUL is available on all systems.

Parameters

A full description of S2PUL, including a diagram of the sequence, is provided in the manual *Getting Started*.

2.12 S2PULR—Standard Two-Pulse in Reverse Configuration

The `s2pulr` macro sets up parameters for a S2PULR pulse sequence. Figure 22 is a diagram of the sequence. The local oscillator (L.O.) signal must be taken from the decoupler board. No decoupling is supported in this sequence.

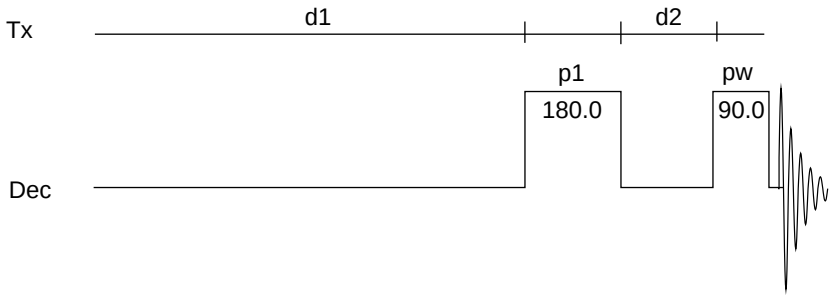


Figure 22. S2PULR Pulse Sequence

Applicability

S2PULR applies to VXR and UNITY systems only.

Parameters

The observe channel uses the decoupler hardware and is controlled by the parameters `dn` (which must be set to `'H1'`), `dof`, `dpwr` (or `dhp`), `p1`, and `pw`.

The macros `movetof` and `movesw` cannot be used with this sequence except in the following way: `tof=dof movetof` (or `movesw`) `dof=tof`.

Technique

1. Acquire a ^{1}H spectrum in the normal manner.
2. Enter **`s2pulr`** to convert the parameter set.

Chapter 3. Multidimensional NMR

Sections in this chapter:

- 3.1 “Interferograms,” this page
- 3.2 “2D NMR Step-by-Step,” page 90
- 3.3 “Phase-Sensitive 2D NMR,” page 91
- 3.4 “Data Acquisition: Arrayed 2D,” page 92
- 3.5 “Weighting,” page 95
- 3.6 “Phasing Before the 2D Transform,” page 97
- 3.7 “Baseline Correction,” page 98
- 3.8 “Processing Phase-Sensitive 2D and 3D Data,” page 101
- 3.9 “2D and 3D Linear Prediction,” page 107
- 3.10 “Phasing the 2D Spectrum,” page 108
- 3.11 “Display and Plotting,” page 109
- 3.12 “Interactive 2D Color Map Display,” page 115
- 3.13 “Interactive 2D Peak Picking,” page 120
- 3.14 “3D NMR,” page 129
- 3.15 “4D NMR Acquisition,” page 134

In some respects, 2D NMR is similar to an arrayed 1D experiment. In both, as a function of time (one of the time variables in the pulse sequence), we obtain a series of FIDs that we then transform to become a series of spectra. For 2D experiments, however, the times for each experiment are *not* explicitly specified. Instead, two new parameters are used: `sw1`, which describes our “2D” spectral width (to be discussed shortly), and `ni`, the number of increments, which sets the number of different experiments we will do. The implicit time variable will then be incremented from experiment to experiment as determined by `sw1`.

3.1 Interferograms

Once the data are obtained and transformed along the acquisition dimension, we have a series of spectra. If this were a 1D arrayed experiment, like an inversion-recovery T_1 experiment, we would see that the peak heights behave exponentially as a function of time. In 2D experiments, however, the peaks heights will oscillate as a function of time, and that oscillation is the information of interest. To unravel this information, we first transpose the matrix to form a series of *interferograms*.

Each interferogram contains a series of points that represent the peak height at a particular frequency in the original spectrum as a function of time. Of course, most of these interferograms contain only noise, because many of the frequencies in the original

spectrum also contained noise. However, some interferograms, namely those corresponding to the peaks in the original spectrum, contain useful information.

The time that is varied in a 2D experiment is known as the *evolution time* or t_1, because it is the first of two key time periods in the 2D experiment. Evolution time is controlled in VNMR software by the parameter `d2`. This time is normally calculated by setting the number of increments to the value of the parameter `ni` and the increment value to `1/sw1`. The value of `ni` determines if a 2D experiment will be run. Initially, `d2` can be set to any value but is usually set to zero.

The `d2` array does not appear in the display `da` (i.e., `d2` is "implicitly" arrayed). Only the first value of `d2` appears as the parameter value in the display `dg`. A minimum of eight increments must be used for `ni` to do a 2D transform. Typical numbers range from 32 to 512. The `addpar('2d')` macro creates the 2D acquisition parameters `ni`, `sw1`, and `phase` (`par2d` functions the same).

The time during which the signal is detected is known as the *detection time* or t_2, because it is the second of the two key time periods. After transform of the signals detected during the time t_2, the "normal" spectrum appears along the f_2 axis. The second transform reveals information about the frequencies of oscillations during the t_1 time period along the f_1 axis.

Many parameters that refer to the new f_1 axis in a 2D experiment are identified by the number 1 (e.g. `sw1`, `lb1`, `fn1`), whereas the normal 1D parameters control f_2.

The process of transformation, transposition to interferograms, and second transformation may seem complicated, however, it can all be reduced to literally a single command, or even a single menu choice, that starts an acquisition of a 2D experiment and performs all the necessary processing when the experiment is done. So the process can be fairly simple.

3.2 2D NMR Step-by-Step

The examples in this section provide an excellent way to get started in 2D NMR. The 2D concepts involved are described in greater detail later in this chapter.

To Process Stored Data

1. In the VNMR menus, click on **Main Menu** > **Workspace**.

   The Workspace menu appears on the lower row of menus.

2. Join the experiment in which you want to perform the 2D processing by clicking on the button for the experiment: Exp2, Exp3, etc. If the experiment does not exist, click on Create New, then click on the button to join the experiment.

3. Enter **`rt('/vnmr/fidlib/fid2d')`**.

   The text window displays the parameters values for the 2D sample data.

4. Click on **Process** > **Select Params** > **Sinebell** > **Large** > **Return**.

   This establishes reasonable initial choices for the processing parameters for your experiment. A number of other choices are available for processing.

5. Click on **Full Transform**.

   This initiates 2D processing. The processing ends with the display of a color map of the 2D frequency data.

6. Adjust the vertical scale `vs2d` and threshold `th` to display the data in an appropriate fashion. Each time you change `vs` using the keyboard, be sure to click the Redraw button to redisplay the color map.
7. To symmetrize the data, enter **`foldt`**.
8. To produce a 2D contour plot, enter **`pcon page`**.

To Acquire a Simple COSY

1. Perform a 1D ^{1}H experiment on a sample of interest. After processing, display two cursors and place them around a region containing only the peaks, omitting most of the baseline region at both ends of the spectrum.

   Certain peaks, such as a small residual $CHCl_3$ peak in a spectrum otherwise containing only aliphatic resonances, can be ignored if they are far enough away not to "fold back into" the spectrum.
2. Enter **`movesw ga`**.

   This moves the spectrum window and then obtains a narrowed spectrum.
3. Use the **`mp(<from,>to)`** command to move the 1D parameters to another experiment. For example, enter `mp(1,4)` if you performed the 1D experiment in `exp1` and you want to perform the 2D experiment in `exp4`. Then join the 2D experiment by entering, for example, `jexp4`.
4. Enter **`cosy`**.

   This is a macro that will modify your parameter set to perform an absolute-value COSY experiment. The total estimated time for the experiment should be displayed on the screen. If you wish, change `nt` and type `time` to alter the conditions. For your first experiment, `nt=16`, `ss=1`, and `d1=1` is recommended.
5. Enter **`wexp='do2d'`**.

   The value of the `wexp` parameter specifies the end of experiment process.
6. Enter **`au`**.

   This starts the acquisition. When the data acquisition is complete, the data is automatically processed and displayed.
7. Adjust the vertical scale `vs` and threshold `th` to display the data in an appropriate fashion. Most of the screen should be black when `vs` and `th` are properly adjusted, with colored spots representing the diagonal and cross peaks. Each time you change `vs` using the keyboard, be sure to enter `dconi` to redisplay the contour plot. The parameter `th` can be adjusted interactively.
8. To symmetrize the data, enter **`foldt`**.
9. To plot the data, enter **`pcon page`**.

3.3 Phase-Sensitive 2D NMR

For many years, 2D NMR experiments were performed and displayed in an absolute- value mode. Just as in 1D NMR, phase-sensitive processing and display offers better sensitivity and resolution. Phase-sensitive 2D NMR by itself is simply the ability to display and plot *phased data*, as opposed to absolute-value data. There are four kinds of experiments in which a user might want to examine phase-sensitive data:

- A 2D experiment in which the data are not expected to appear in absorption mode in both directions, but in which it is nonetheless desirable to observe the data in a phase-sensitive presentation.
- A 2D experiment in which the data, processed in a suitable way, *are* expected to appear in absorption mode in both directions. Heteronuclear 2D-J is such an experiment.
- An experiment in which two different experiments are performed for each value of t_1, typically using different phase cycles, producing a full complex data set for the second transformation. We shall refer to this method, popularized by States, Haberkorn, and Ruben (*J. Magn. Reson.* **1982**, *48*, 286), as the *hypercomplex method*
- An experiment in which the phase of the excitation pulse is incremented as a function of t_1 (TPPI or Time Proportional Phase Incrementation, see Marion and Wuthrich, *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967) and which produces real data along the t_1 axis.

Each of the experiments referred to above can be performed and processed with the VNMR software. Complex transforms are usually performed along t_1, which is the ideal situation for the hypercomplex method. TPPI data can be processed along t_1 with either a complex FT or a real FT, depending upon the method of data collection.

In general, the hypercomplex method is the method of choice using VNMR software. A natural first reaction, since this method requires *two* data tables instead of one, is to assume that it requires twice as much storage as the TPPI method. This is untrue, however, for the same reason that a real 1D transform covering a given spectral width requires exactly as much data as a complex 1D transform— the sampling rate must be twice as high in the real case to produce the same result. In the same way, the TPPI method requires only one data table, but requires sampling to occur twice as frequently along t_1, thereby incurring twice the data size per data table to produce the same real resolution. So in this sense the two experiments are equivalent in data storage requirements and experimental time.

3.4 Data Acquisition: Arrayed 2D

2D experiments have one implicitly “arrayed” parameter, `d2`. Like 1D experiments, however, 2D experiments can also have other parameters explicitly arrayed. This feature can be used, of course, for purposes that have nothing to do with phase-sensitive 2D, such as running a series of 2D-NOE experiments using different mixing times. As we shall see below in discussing the processing of such data, this feature alone opens up a variety of experiments, including addition/subtraction of two or more 2D experiments. Table 16 lists commands and parameters associated with arrayed 2D and 3D data acquisition.

Hypercomplex Method

The hypercomplex method of phase-sensitive 2D NMR requires the use of two data tables. Appropriate pulse sequences must be created (see below for more details of this point) which, as a function of some parameter, generate a different sequence of pulses or pulse phases suitable for generating the two component experiments of the hypercomplex method.

Any parameter may be used for this purpose. As a convention, we use the parameter `phase`, which takes on values of 0, 1, or 2:

- A value of `phase=0` can be used to produce a phase cycle suitable for a conventional (non-phase-sensitive) 2D experiment.

Table 16. Arrayed 2D & 3D Data Acquisition Commands and Parameters

Commands	
`addpar('2d'|'3d')`	Add 2D or 3D parameters to the current experiment
`par2d`	Create 2D acquisition parameters
`wft2d*`	Weight and Fourier transform 2D data
`* wft2d<(<options,>coefficients)>`	
Parameters	
`d2*`	Incremented delay for 1st indirectly detected dimension
`d3` {number, in seconds}	Incremented delay for 2nd indirectly detected dimension
`ni` {number}	Increments in 1st indirectly detected dimension
`ni2` {number}	Increments in 2nd indirectly detected dimension
`phase` {number}	Phase selection
`phase2` {number}	Phase selection for 3D acquisition
`sw1` {100 to 100000, in Hz}	Spectral width in 1st indirectly detected dimension
`sw2` {100 to 100000, in Hz}	Spectral width in 2nd indirectly detected dimension
* `d2` {0 to 4095,in sec} *GEMINI 2000*, {0 to 8190, in sec} other systems	

- Running instead an array of experiments with `phase=1,2` produces the two experiments suitable for the hypercomplex method.

You may ascertain the possible values of `phase` by reading the source code in the `psglib` directory for any particular pulse sequence.

TPPI Method

The TPPI method of phase-sensitive 2D NMR requires one data table when `phase=3`. The data must be processed along t_1 with a complex Fourier transform by setting `proc1` (which sets the type of data processing to be performed on the t_1 interferogram) to `'ft'`. This manner of implementing TPPI leads to a doubling of the f_1 frequency axis.

When an arrayed 2D experiment is run in this manner, there is in reality a double array: `d2` (the evolution time) and `phase`. The order of these arrays is such that the `phase` array is cycled the most rapidly, so that the order of the experiments is, for example:

```
d2=0          phase=1
d2=0          phase=2        States-Haberkorn
d2=1/sw1      phase=1
d2=1/sw1      phase=2

d2=0          phase=3
d2=1/sw1      phase=3        TPPI (non-arrayed)
d2=2/sw1      phase=3
d2=3/sw1      phase=3
```

Not all pulse sequences have the TPPI method incorporated.

When an experiment is in progress, the acquisition status window displays a count of the current FID and the number of completed transients (`ct`) in that FID. As indeed happens with a 1D arrayed experiment, the current FID number is actually the *total* count of the completed FIDs to this point, including all arrays. Since the `phase` parameter is cycling the most rapidly, and since typically `phase` is an array of two values, the current FID number is typically *twice* the number of the current increment. For example, when the counter reads FID 54, this means that 27 FIDs of the first type of experiment have been

completed, 26 of the second type, and the system is working on the 27th experiment of the second type.

Real-Time 2D

VNMR software can perform *real-time 2D*, that is, 2D actions while the experiment is still in progress. Once eight or more increments have been completed, you can enter the `wft2d` command to perform the full 2D transform on the data that exists up to that point.

For some experiments, such as heteronuclear chemical shift correlation and homonuclear 2D-J experiments, you will be surprised at how few increments are needed to resolve the resonances of interest. Others may require more increments. In any case, if you find you have sufficient data to solve the problem, you can always abort the experiment, so that the remaining increments are not performed, and proceed to the next problem.

Macros for 2D Experiments

Table 17 lists pulse sequences for 2D experiments that have a macro on the system to help you set up the experiment.

Table 17. Macros for 2D Experiments

Macros	
`cosy`	Set up COSY, correlated spectroscopy
`cosyps`	Set up COSYPS, phase-sensitive COSY
`dqcosy`	Set up DQCOSY, double-quantum filtered COSY
`het2dj`	Set up HET2DJ, heteronuclear 2D-J experiment
`hetcor<(exp_num)>`	Set up HETCOR, heteronuclear chemical shift correlation
`hmqc<(isotope)>`	Set up HMQC, reverse detection heteronuclear multiple quantum
`hmqcr`	Set up HMQCR, HMQC with “reverse configuration”
`hom2dj`	Set up HOM2DJ, homonuclear J-resolved 2D experiment
`inadqt**`	Set up INADEQUATE experiment
`mqcosy<(level)>`	Set up MQCOSY, multiple quantum filtered COSY
`noesy`	Set up NOESY, laboratory frame Overhauser experiment
`relayh`	Set up absolute valueCOSY or single or double RELAY-COSY
`roesy<(ratio)>**`	Set up ROESY, rotating frame Overhauser experiment
`tncosyps*`	Set up TNCOSYPS, COSYPS with water suppression
`tndqcosy*`	Set up TNDQCOSY, DQCOSY with water suppression
`tnmqcosy*`	Set up TNMQCOSY, MQCOSY with water suppression
`tnnoesy*`	Set up TNNOESY, NOESY with water suppression
`tnroesy*`	Set up TNROESY, ROESY with water suppression
`tntocsy*`	Set up TNTOCSY, TOCSY with water suppression
`tocsy**`	Set up TOCSY, total correlation spectroscopy

* Not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems
** Not available on *GEMINI 2000* system

Each macro displays suggestions on how to select values for the parameters in that experiment. Complete listings of each pulse sequence are found on your system disk in the directory `/vnmr/psglib`. Note that the INADEQUATE, MQCOSY, ROESY, and TOCSY sequences are not available on *GEMINI 2000* systems. None of the `tn` sequences are available on *GEMINI 2000*, *MERCURY-VX*, or *MERCURY* systems.

For further information, including diagrams and literature references, see Chapter 4, “Multidimensional and Advanced Experiments,” .

3.5 Weighting

This section describes weighting functions for processing in the ni and ni2 dimensions.

Parameters

Each 2D weighting parameter (the parameters ending with "1") listed in Table 18 is analogous to a similarly named parameter for 1D experiments listed the manual *Getting Started* (the same parameter name but without the 1).

Table 18. Weighting Parameters for ni and ni2 Dimensions

Parameters	
awc1 {'n', number}	Additive weighting const. in 1st indirectly detected dimension
awc2 {'n', number}	Additive weighting const. in 2nd indirectly detected dimension
fn1 {'n', number}	Fourier number in 1st indirectly detected dimension
fn2 {'n', number}	Fourier number in 2nd indirectly detected dimension
gf1 {'n', number in sec}	Gaussian function in 1st indirectly detected dimension
gf2 {'n', number in sec}	Gaussian function in 2nd indirectly detected dimension
gfs1 {'n',number in sec}	Gaussian shift constant in 1st indirectly detected dimension
gfs2 {'n',number in sec}	Gaussian shift constant in 2nd indirectly detected dimension
lb1 {'n',number in Hz}	Line broadening in 1st indirectly detected dimension
lb2 {'n',number in Hz}	Line broadening in 2nd indirectly detected dimension
sb1 {'n',number in sec}	Sinebell constant in 1st indirectly detected dimension
sb2 {'n',number in sec}	Sinebell constant in 2nd indirectly detected dimension
sbs1 {'n',number in sec}	Sinebell shift constant in 1st indirectly detected dimension
sbs2 {'n',number in sec}	Sinebell shift constant in 2nd indirectly detected dimension
wtfile1 {",file}	User-defined weighting in 1st indirectly detected dimension
wtfile2 {",file}	User-defined weighting in 2nd indirectly detected dimension

Parameters for 2D experiments are used for processing the t_1 domain (the interferograms) or ni (first indirectly detected) dimension. Parameters ending with a "2" are used for processing the ni2 (second indirectly detected) dimension.

In non-phase-sensitive (absolute-value and power) 2D experiments, "pseudo-echo," sinebell, or sinebell-squared weighting is typically used to attenuate long dispersion tails. This weighting is often responsible for a significant loss in sensitivity in such 2D experiments.

In phase-sensitive 2D experiments, the key in using weighting functions is to ensure that the weighted FID or interferogram decays to zero by the end to avoid "truncation wiggles." The Gaussian function (gf and gf1 parameters) is ideally suited for this; typical values might be gf=0.6*at, gf1=0.6*ni/sw1 (=0.6*at1). Resolution enhancement (using negative lb or negative lb1) may be helpful in cases of spectral overlap, but can also be dangerous, since the "dips" that it can induce around the sides of peaks show up as peaks of opposite sign in the 2D plot, complicating analysis.

Setting Values

Setting values for 2D weighting parameters can be done through various macros and an interactive program (described in the next section). Table 19 lists these tools.

The following macros are available to generate values for 2D weighting parameters:

Table 19. Commands and Macros for Setting 2D Weighting Values

Commands	
`pseudo<(C1,C2,C3,C4)>`	Set parameters for pseudo-echo weighting
`sine<(shift<,npoints<,domain>)>`	Find values for a sine window function
`sinebell`	Select default parameters for sinebell weighting
`sinesq<(shift<,npoints<,domain>)>`	Find values for a sine-squared window function
`wti<(element_number)>`	Interactive weighting
`wtia<(element_number)>`	Interactive weighting for 2D absorptive data
Macros	
`gaussian`	Set up unshifted Gaussian window
`pi3ssbsq`	Set up pi/3 shifted sinebell-squared window
`pi4ssbsq`	Set up pi/4 shifted sinebell-squared window
`sqcosine`	Set up unshifted cosine-squared window
`sqsinebell`	Set up unshifted sinebell-squared window

- `gaussian` sets up an unshifted Gaussian window function in 1, 2, or 3 dimensions. This macro checks whether data is 1D, 2D, or 3D. The argument `t1_inc` is the number of t1increments; the default is `ni`. The argument `t2_inc` is the number of t2 increments; the default is `ni2`.
- `pi3ssbsq` and `pi4ssbsq` are macros that respectively set up pi3 and pi4 shifted sinebell-squared window functions in 1, 2, or 3 dimensions and check whether data is 1D, 2D, or 3D. Both macros use the `t1_inc` and `t2_inc` arguments, described in the previous description of the `gaussian` macro.
- `pseudo<(C1,C2,C3,C4)>` generates an initial guess at good values for `lb`, `gf`, `lb1`, and `gf1` for absolute-value 2D experiments. To generate modified guesses, four coefficients (described in the *VNMR Command and Parameter Reference*) are available.
- `sinebell` sets `sb` and `sb1` to one-half the acquisition time. Other weighting is turned off. Use `sinebell` in absolute-value 2D experiments only.
- `sine(<shift><,npoints><,domain>)` calculates appropriate values for `sb` and `sbs` (if argument `domain` is `'f2'`) or for `sb1` and `sbs1` (if `domain` is `'f1'`) to achieve a sine window function. If `shift` is greater than 0, the starting value for the window function is given by `sine(pi/shift)`; otherwise, the starting value is 0. `npoints` specifies the number of real points the window function spans. If `domain` is not specified, the value of the parameter `trace` is used as the default.
- `sinesq(<shift><,npoints><,domain>)` calculates appropriate values for `sb` and `sbs` (if `domain='f2'`) or for `sb1` and `sbs1` (if `domain='f1'`) to achieve a sine squared window function. The arguments are used the same as the `sine` command.
- `sqcosine` and `sqsinebell` are macros that respectively set up unshifted cosine-squared and sinebell-squared window functions in 1, 2, or 3 dimensions and check whether data is 1D, 2D, or 3D. Both macros use the `t1_inc` and `t2_inc` arguments, described in the previous description of the `gaussian` macro.

Interactive Weighting

The `wti<(element_number)>` command allows interactive setting of weighting parameters for both t_2 FIDs and t_1 interferograms (both the `ni` and `ni2` dimension). The optional argument `element_number` specifies which FID element or interferogram

trace is to be used in adjusting the weighting parameters. The default value is the currently active element or trace. `wti` responds appropriately to `phfid` and `lsfid` for t_2 FIDs, `phfid1` and `lsfid1` for t_1 interferograms defined by `ni`, and `lsfid2`, and `phfid2` for t_1 interferograms defined by `ni2`.

The following parameters are used with interactive weighting:

- `awc`, `awc1`, and `awc2` set the additive weighting constant; added in to the weighting function after the `lb` and `sb` (`sbs`) contributions but before the `gf` (`gfs`) contributions.
- `gf`, `gf1`, and `gf2` set the Gaussian apodization constant, in seconds.
- `gfs`, `gfs1`, and `gfs2` set the Gaussian function shift, in seconds. This shifts the origin of the Gaussian function; active only if `gf` (or `gf1`) is active.
- `lb`, `lb1`, and `lb2` set the line broadening factor, in Hz; a positive value gives sensitivity enhancement; a negative value gives resolution enhancement.
- `sb`, `sb1`, and `sb2` set the sinebell time period, in seconds; a negative value gives a sine squared bell.
- `sbs`, `sbs1`, and `sbs2` set the sinebell shift, in seconds; shifts the origin of the sinebell; active only if `sb` (or `sb1`) is active.

These parameters can be typed in or changed with the left mouse button in the proper field. The right mouse button turns off the spectrum for a faster response to changes in the weighting function.

The `wtia<(element_number)>` command allows the same weighting parameters to be set interactively for 2D absorptive data. The argument `element_number` is used the same as in the `wti` command.

3.6 Phasing Before the 2D Transform

Table 20 summarizes the commands and parameters discussed in this section.

For a phase-sensitive 2D display, only the `ph` (phase-sensitive along f_2) command and the `ph1` (phase-sensitive along f_1) command are relevant to ensure that phasing either is performed during the 2D FT or can be performed after the 2D FT along each dimension. `ph` and `ph1` should be executed according to the following rules:

- `pmode=''` is both `ph` and `ph1` must be executed before performing 2D FT.
- `pmode='partial'` is only `ph` must be executed before performing 2D FT.
- `pmode='full'` is either command must be executed before 2D FT.

To obtain pure 2D absorptive lineshapes requires a properly phased spectrum in the first dimension, that is, along f_2. To facilitate this operation, the `wft` and `ft` programs (actually the same program) allow normal 1D transforms on 2D data. After an experiment is complete, or while it is in progress, type `wft(1)` or `ft(1)`. The first FID will be transformed. After the transform, the spectrum will be displayed. Phase this spectrum in the usual way. If `pmode='full'`, all phasing along f_2 can be performed after the 2D FT if corrections in f_2 phasing are required. To prevent automatic spectral display (`ds`), type `wft('nods')`.

One subtle point remains. In, for example, a COSY experiment acquired using the hypercomplex method, the first spectrum of the first array element contains data resulting from the summing of the signal from 90_x–90_x and 90_x–90_{-x} pulse sequences. But both of these experiments produce in principle *no* signal in the *xy* plane! In this case, phasing is

Table 20. Commands and Parameters for Phasing Before the 2D Transform

Commands	
`ds*`	Display a spectrum
`ft*`	Fourier transform 1D data
`ft2d*`	Fourier transform 2D data
`ph`	Set phased mode along directly detected dimension
`ph1`	Set phased mode along 1st indirectly detected dimension
`wft*`	Weight and Fourier transform 1D data

```
* ds<(index)>, ds<(options)>
  ft<(<options,><'nf'><,start><,finish><,step>)>
  ft('inverse',exp_number,expansion_factor),
  ft2d(array_element)
  ft2d<(<options,><plane_number,><coefficients>)>,
  ft2d('ni'|'ni2',element_number,increment),
    ('ni'|'ni2',increment,<coefficients>)
  wft<(<options,><'nf'><,start><,finish><,step>)>,
  wft('inverse',exp_number,expansion_factor)
```

Parameters	
`lp1` {–3600 to 3600, in deg}	First-order phase in 1st indirectly detected dimension
`lp2` {–3600 to 3600, in deg}	First-order phase in 2nd indirectly detected dimension
`pmode` {'','partial','full'}	Processing mode for 2D data
`rp1` {–360 to 360, in deg}	Zero-order phase in 1st indirectly detected dimension
`rp2` {–360 to 360, in deg}	Zero-order phase in 2nd indirectly detected dimension

performed on the first spectrum of the *second* experiment, which is displayed by `ds(2)`. Different experiments may require different first domain phasing procedures. With the standard pulse sequences, both array elements can be phased for pure absorption simultaneously. For cases where one data set is “in phase” and the other is “out of phase” for a given set of phase parameters (`lp` and `rp`), the `ft2d` program can be instructed to extract the properly phased data from each experiment.

After the full transform, f_1 phasing is possible using the `lp1` and `rp1` parameters if `pmode` has been set to `'partial'` or `'full'`. f_1 phasing for the `ni2` dimension is done using the parameters `lp2` and `rp2`. Once satisfactory f_1 and f_2 phasing has been obtained, future retransforms may be done with `pmode=''` (two single quotes with no space between the quotes). This results in a faster initial display of the processed data.

3.7 Baseline Correction

Table 21 lists commands and parameters covered in this section.

The `ft` and `ft2d` commands (and related commands) multiply the first point of each FID by `fpmult` (the default value is 1.0, except that if the processing involves backward extension of the time-domain data with linear prediction, the default value is then 0.5) and the first point of each interferogram by `fpmult1` (default value is 0.5) for the `ni` dimension or `fpmult2` (default value is 0.5) for the `ni2` dimension. `fpmult` attempts to compensate for the first point distortion caused by analog filters (see Otting, Widmer, Wagner and Wüthrich, *J. Magn. Reson.* **1986**, *66*, 187).

The effect of using the `fpmult` is to perform a linear baseline correction on all f_2 data, reducing negative-going ridges along f_2 in phase-sensitive 2D data.This correction is not needed in experiments such as COSY where the FID *starts* at zero and grows or in absolute-

Table 21. Baseline and Drift Correction Commands and Parameters

Commands	
`bc*`	2D baseline correction
`calfa`	Recalculate alfa so that the left phase is zero
`cdc`	Cancel drift correction
`cfpmult`	Calculate first-point multiplier for 2D experiments
`crof2<(alfa)>`	Recalculate rof2 so that lp=0
`dc`	Calculate spectral drift correction
`dc2d('f1'|'f2')`	Apply drift correction to 2D spectra
`ft*`	Fourier transform 1D data
`ft2d*`	Fourier transform 2D data
`wft*`	Weight and Fourier transform 1D data

`* bc(trace_direction<,order><,trace_start><,trace_end>)`
`ft<(<options,><'nf'><,start><,finish><,step>)>`
`ft('inverse',exp_number,expansion_factor),ft2d(array_element)`
`ft2d<(<options,><plane_number,><coefficients>)>,`
`ft2d('ni'|'ni2',element_number,increment),`
`ft2d('ni'|'ni2',increment,<coefficients>)`
`wft<(<options,><'nf'><,start><,finish><,step>)>,`
`wft('inverse',exp_number,expansion_factor)`	

Parameters	
`alfa` {0 to 1e8, in µs}	Set alfa delay before acquisition
`dcg` {'dc', 'cdc'}	Drift correction group
`fpmult` {'n', number}	First point multiplier for np FID data
`fpmult1` {'n', number}	First point multiplier for ni interferogram data
`fpmult2` {'n', number}	First point multiplier for ni2 interferogram data
`lp` {–3600 to 3600, in deg}	First-order phase along directly detected dimension
`rof2` {0 to 8190, in ms}	Receiver gating time following pulse

value mode presentation if pseudo-echo or sinebell processing is used, because the processing function goes to zero at t_2=0, forcing all FIDs to start at zero amplitude.

Unless `lp` is approximately zero, `fpmult` will affect both the `dc` offset and the curvature of the spectrum during 2D data processing. Obtaining a trial spectrum and phasing it to pure absorption will provide from the spectrum the current values of the parameters `alfa` and `lp`. Using these parameters, the `calfa` macro can calculate a new value for `alfa` so that `lp` is rendered approximately 0.

The `crof2` macro recalculates a new value for `rof2` (receiver gating time following a pulse) based upon the current `rof2` and `lp` (first-order phase) values, so that `lp` is rendered approximately 0. For `crof2` to work properly, a trial spectrum must be obtained and phased to pure absorption. This spectrum provides the current `rof2` and `lp` values for `crof2`. The value of the `alfa` delay is left constant, provided `rof2` does not become less than 1 µs.

First-Point Multiplier

The best value of `fpmult` is a function of the filter setting and should be determined empirically. It can be determined before, during, or after the 2D experiment by using `wft(1)`.

1. With a properly phased first increment spectrum on the screen, enter `dc`.

2. Position the mouse-controlled arrow at the right edge of spectrum baseline (to keep track of the ideal baseline position).
3. Enter `cdc` and observe the new position of the baseline. It typically drops.
   - If the baseline goes negative, set `fpmult` to greater then 1.0 (try 1.5) and enter `wft(1)`.
   - If the baseline rises but does not return to the position indicated by the mouse arrow, increase the value of `fpmult` and enter `wft(1)` again. If in doing so the baseline rises above the ideal level, reduce `fpmult` and try again.

   Only a few tries are required before the proper value of `fpmult` is found.

The macro `cfpmult` is provided that does the procedure above automatically. This applies only to data in t_2. No equivalent macro for t_1 data is provided. Normally, no correction for `fpmult1` is necessary.

Setting `fpmult='n'` and `fpmult1='n'` disables these features of the `ft` and `ft2d` programs. This would be the usual value for sinebell or pseudo-echo processing.

Baseline Correction

An alternative to the use of `fpmult` is the use of the baseline correction command `bc`. The implementation of `bc` in 2D processing uses the spline or second to twentieth order polynomial fitting of predefined baseline regions. These regions are set up prior to the use of `bc` by setting integral resets (set `intmod='partial'`) so that integrals appear only over regions of the spectrum with signals present. These may be set after a `wft(1)`, as described in the manual *Getting Started*. The quality of the baseline correction may be assessed by `bc(1)`. In setting baseline regions near the ends of the spectrum, the `bc` operation does essentially the equivalent of `fpmult` because this represents a simple dc correction.

If this mode is used, the `wft2d` command must have an argument `'bc'` and an optional order value such as `wft2da('bc',1)`. After the first (t_2) transform, the `bc` program executes, and the interferograms are then calculated from the baseline-corrected data. After the second transform, `bc` may be used again, this time along f_1 using the command `bc('f1')`. Make sure that the resets are appropriate. They will automatically be so if `sw=sw1` in a homonuclear experiment. Of course, in a heteronuclear shift correlation experiment no proper reset points may be set, in general.

FID Drift Correction

A `dc` offset in time-domain data transforms into a “center glitch” in the frequency spectrum. For 1D data, the `ft` program automatically applies a `dc` correction to the FID. Such a correction is not applied to 2D FIDs or interferograms unless explicitly requested. The command `ft2d('t2dc')` causes a `dc` correction to be applied to each t_2 FID before the first FT and `ft2d('t1dc')` causes a `dc` correction to be applied to each t_1 interferogram prior to the second FT. In both cases, the last one-sixteenth of the time-domain data is used to calculate the `dc` correction.

The `dcg` parameter contains the results of the `dc` or `cdc` command. This parameter cannot be set in the usual way but it can be queried (`dcg?`) to determine whether drift correction is active.

Spectral Drift Correction

The command `dc2d('f1'|'f2')` is the 2D equivalent of `dc` and is run only after the 2D transform. Use `dc2d('f1')` for corrections along f_1 and `dc2d('f2')` for corrections along f_2. The drift correction calculation is done separately for each trace in the 2D data set.

3.8 Processing Phase-Sensitive 2D and 3D Data

After 2D data has been acquired, the complete 2D transformation can be performed with a single command, with or without weighting as appropriate. Table 22 lists commands and parameters associated with this processing.

The general flow of information during the process of phase-sensitive 2D transformation is depicted in Figure 23 for `pmode='partial'`.

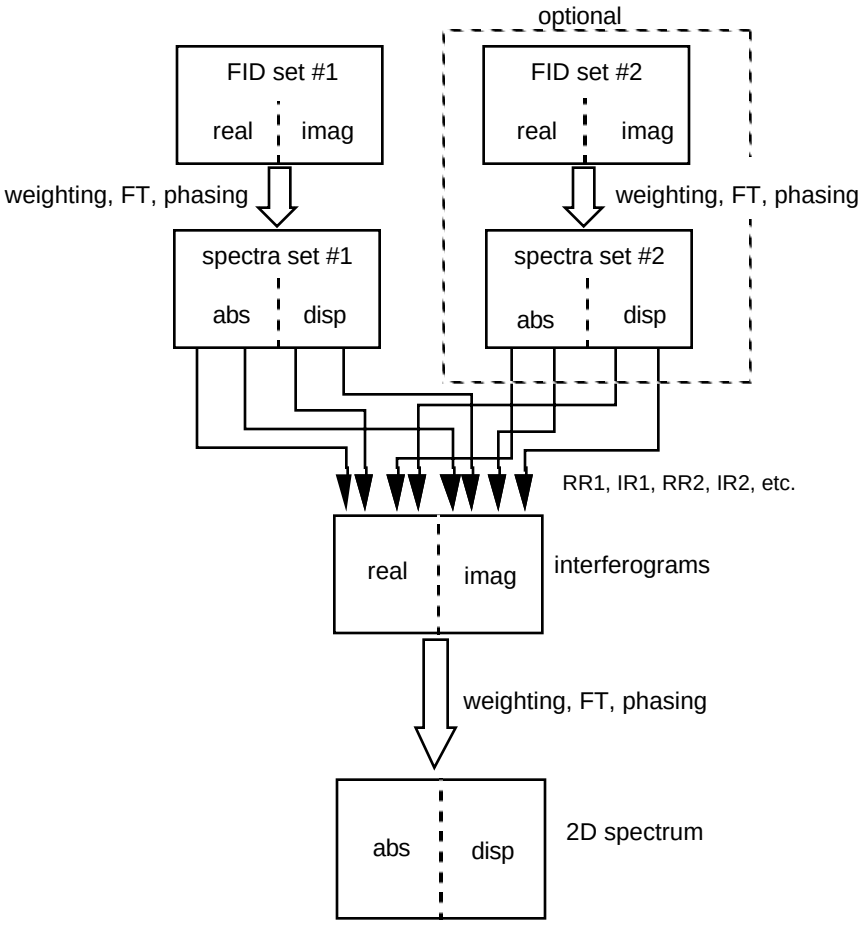


Figure 23. Data Flow in Phase-Sensitive 2D Transformation

A series of complex FIDs, obtained as a function of t_1, are transformed to become a series of spectra. Each spectrum consists of a real and imaginary part. Each spectrum is then phase rotated, according to the phase correction determined from an individual spectrum. We now have a series of spectra, each consisting of an absorptive and a dispersive part, formed as linear combinations of the original real and imaginary parts. Complex interferograms are

Table 22. Tools for Processing Phase-Sensitive 2D and 3D Data

Commands	
`addpar('2d'|'3d')`	Add selected 2D or 3D parameters to current experiment
`dg2`	Display group of 3rd and 4th channel/3D parameters
`foldt<('symm'|'triang')>`	Fold COSY-like spectrum along diagonal axis
`ft1d*`	Fourier transform of 2D data
`ft1da<(options)>`	Fourier transform "halfway" for pure absorption 2D data
`ft2d*`	Fourier transform 2D data
`ft2da<(options)>`	Fourier transform for pure absorption 2D data
`ft3d*`	Perform a 3D FT on a 3D FID data set (VNMR, UNIX)
`par3d`	Create 3D acquisition, processing, display parameters
`parlp`	Create parameters for linear prediction
`wft1d*`	Weight and Fourier transform f_2 for 2D data
`wft1da<(options)>`	Weight and FT "halfway" for pure absorption 2D data
`wft2d*`	Weight and Fourier transform 2D data
`wft2da<(options)>`	Weight and Fourier transform for pure absorption 2D data

`* ft1d(element_number), ft1d<(<options,><coefficients>)>`
`ft2d<(<options,><plane_number,><coefficients>)>,`
`ft2d('ni'|'ni2',element_number,increment)`
`ft2d('ni'|'ni2',increment,<coefficients>)`
`ft3d<(<data_dir><,number_files><,'nocoef'><,plane_type>)>  (VNMR)`
`ft3d –e exp_number –f –r <options>  (UNIX)`
`wft1d(element_number), wft1d<(<options,><coefficients>)>`
`wft2d<(<options,>coefficients)>`	

Parameters	
`daslp`	Increment for t1 dependent first-order phase correction
`fn` {'n',number}	Fourier number along directly detected dimension
`fn1` {'n',number}	Fourier number in 1st indirectly detected dimension
`fn2` {'n',number}	Fourier number in 2nd indirectly detected dimension
`intmod` {'off','partial','full'}	Integral display mode
`lsfid` {'n',number}	Number of complex points to left shift np FID
`lsfid1` {'n',number}	Number of complex points to left-shift ni interferogram
`lsfid2` {'n',number}	Number of complex points to left-shift ni2 interferogram
`lsfrq` {number, in Hz}	Frequency shift of the fn spectrum
`lsfrq1` {number, in Hz}	Frequency shift of the fn1 spectrum
`lsfrq2` {number, in Hz}	Frequency shift of the fn2 spectrum
`pmode` {','partial','full'}	Processing mode for 2D data
`proc` {'ft', 'rft', 'lp'}	Type of processing on np FID
`proc1` {'ft', 'rft', 'lp'}	Type of processing on ni interferogram
`proc2` {'ft', 'rft', 'lp'}	Type of processing on ni2 interferogram
`sw` {100 to 100000, in Hz}	Spectra width
`sw1` {100 to 100000, in Hz}	Spectral width in 1st indirectly detected dimension
`sw2` {100 to 100000, in Hz}	Spectral width in 2nd indirectly detected dimension

then formed out of corresponding points along the frequency axis from each of the spectra, and transformed to produce the final 2D spectrum.

The eight lines with arrows in the center of Figure 23 represent coefficients used during the process of transformation. The real and imaginary part of the interferograms can be formed from any linear combination of the real and imaginary parts of one or more spectral sets after the first Fourier transformation. We shall refer to these coefficients below according to the following scheme: `RR1` is the coefficient used to multiply the real part (first `R`) of spectra in set `1` (the `1`) before it is added to the real part (second `R`) of the interferogram.

`IR2` would thus represent the contribution from the imaginary part of spectra in set 2 to the real part of the interferogram, and so on.

For `pmode='full'`, another set of complex interferograms are formed from these two sets of f_2 spectra. This set of interferograms is 90° out-of-phase in f_2 to the previous set and can be constructed without any *additional* coefficients.

Different experiments will require different coefficients. Some, such as heteronuclear 2D-J experiments, consist of only one FID and spectral set, and hence there will be a total of four coefficients. Others, including hypercomplex 2D experiments, will consist of two original data sets and hence a total of eight coefficients. Other experiments are possible with three or even more data sets, requiring in each case four times as many coefficients as the number of data sets (see the macro `wft2dac`).

If there are *n* data sets to be transformed, as in typical phase-sensitive experiments, 4*n* coefficients must be supplied. The first 2*n* coefficients are the contributions to the real part of the interferogram, alternating between real and imaginary parts of the successive data sets. The next 2*n* coefficients are the contributions to the imaginary part of the interferogram, in the same order.

Thus, using the definition that the first letter refers to the source data set, the second letter refers to the interferogram, and the number identifies the source data set, we have the cases shown in the table on the right.

Data Sets	*Coefficient Order*
1	RR1, IR1, RI1, II1.
2	RR1, IR1, RR2, IR2, RI1, II1, RI2, II2.
3	RR1, IR1, RR2, IR2, RR3, IR3, RI1, II1, RI2, II2, RI3, II3.
`...`	...

The coefficients are generally 1, 0, or –1, but other coefficients are acceptable. Any *real* coefficient can be used, and as many coefficients can be non-zero as is desired. Up to 32 coefficients can be supplied, which at four per data set allows the addition, subtraction, etc., of eight 2D data sets (that is, eight different phase cycles). See the macro `wft2dac` for more information.

Processing Programs

A number of processing programs are available:

- `ft1d(coefficients)` performs only the first Fourier transformation along the f_2 dimension (without weighting) and matrix transposition, allowing the display of interferograms with the `wti`, `dcon`, and `dconi` commands.
- `wft1d(coefficients)` functions the same as `ft1d` except weighting is included.
- `ft2d<(<option,>coefficients)>` performs a complete transformation in 2D, without weighting, after 2D data has been acquired. If the first Fourier transformation has already been done using `ft1d`, `wft1d`, `ft1da`, or `wft1da`, then `ft2d` performs only the second (t_1) transform. `'ptype'` or `'ntype'` can be used as the first argument to select P-type or N-type peak selection. The `coefficients` argument are discussed below.
- `wft2d<(<option,>coefficients)>` performs the same as `ft2d` except weighting is included. To perform a normal 2D transform on the `n`-th element in an arrayed 2D experiment, type `wft2d(n)`.
- `ft2da<('bc',polynomial_order)>` runs complete phase-sensitive Fourier transform after the 2D FID data has been acquired. `'bc'` is a keyword to perform a

baseline correction on the f_2 spectra prior to the Fourier transform along f_1. `polynomial_order` is the order of the polynomial used in the baseline correction.

- `wft2da<('bc',polynomial_order)>` functions the same as `ft2da` except weighting is included.
- `ft1da` functions the same as `ft2da` except a Fourier transform along f_1 is omitted.
- `wft1da` functions the same as `ft1da` except weighting is included.

For some 2D data sets, you can save much time by selectively transforming the t_1 interferograms. `ft2d('f2sel')` allows only preselected f_2 regions to be transformed along t_1; the t_1 interferograms in the non-selected f_2 regions are zeroed but *not* transformed. The same mechanism used to select baseline regions for baseline correction (`bc`) is used to select the f_2 regions that are to be transformed along t_1. Set `intmod='partial'` and partition the integral of the spectrum into several regions. The even numbered f_2 regions, e.g., 2, 4, etc., will be transformed along t_1; the odd numbered ones will not be transformed along t_1.

Unreliable peak heights can be caused by Fourier transformation of truncated time-domain data, instead of Fourier numbers `fn` and `fn1` being too low, as might be intuitively expected. To obtain properly defined signals, take one of the following steps:

- Collect data until the signal has decayed to zero in the time domain, or
- Transform the data with zero-filling (`fn>=2*np`, `fn1>=4*ni`).

Taking one of these steps is particularly important in 2D spectra with antiphase or dispersive signals, where underdigitization can lead to signal cancellation.

Common Coefficients for wft2d Processing

A magnitude-mode transform, in which the real part of the interferogram is formed from the real part of the spectra and the imaginary part of the interferogram is formed from the imaginary part, would require `wft2d(1,0,0,1)`. Changing the sign of the imaginary part of the interferogram serves to change the effective direction of the f_1 frequency axis, as is required for data in which N-type peaks are detected. This can be done with `wft2d(1,0,0,-1)`.

In some experiments, including heteronuclear 2D-J, the basic data are purely amplitude modulated, with a starting amplitude of +1. After the first transformation and phasing operation are complete, the dispersion part of each spectrum serves only to produce a phase-twist in the final spectrum without contributing any information. Setting the imaginary part of the second transform to zero produces a pure absorption display in both domains. To do this, type `wft2d(1,0,0,0)` or `ft2d(1,0,0,0)`.

In the hypercomplex method for pure absorption 2D data, we have two complete sets of spectra and must therefore provide eight coefficients to specify the composition of the interferograms. A typical execution of the method described by States, Haberkorn, and Ruben, assuming that the first spectrum of the first data block has been phased for absorption, requires `wft2d(1,0,0,0,0,0,1,0)` to produce pure absorption spectra. For this coefficient set, the standard macro `wft2da` (or `ft2da`) is supplied; thus, in the most common case, you do not need to enter coefficients but simply type `wft2da.`

Other manipulations of two data blocks are formatted similarly. A magnitude-mode 2D experiment that is the sum of the two different experiments can be constructed by `wft2d(1,0,1,0,0,1,0,1)`. For a COSY experiment, this would produce the P-type experiment. Subtracting data block two from block one, which for a COSY experiment gives the N-type COSY, would be accomplished by `wft2d(1,0,–1,0,0,1,0,–1)`. Thus

two different absolute-value 2D experiments (P-type and N-type), and a phase-sensitive 2D experiment, can all be produced from the *same* data set, without acquiring the data again.

Different combinations of data sets with appropriate phase cycling might allow selection of various quantum orders in a *single* experiment. Note that since the coefficients may be different from one, it is possible essentially to phase shift each experiment *separately* (phase shift the receiver) *after* the experiment is done. For TPPI data with `phase=3`, only one data set is collected, and the imaginary part of the second transform is set to zero: `wft2d(1,0,0,0)`.

The parameters `proc` and `proc1` can be used to select the type of processing to be performed along t_2 and t_1, respectively. `proc` (or `proc1`) accepts the value `'ft'` (complex FT, the default if `proc` or `proc1` is not defined), `'rft'` (real FT), or `'lp'` (linear prediction processing on complex data):

- All Varian data and simultaneously sampled Bruker FID data should be processed with a complex FT along t_2, i.e., `proc='ft'`.
- Sequentially sampled Bruker FID data should be processed with a real FT along t_2, i.e., `proc='rft'`.
- Varian hypercomplex (`phase=1,2`) and standard TPPI (`phase=3`) 2D FID data should be processed along t_1 with `proc1='ft'`.
- If 2D Bruker data is converted with the `convertbru` program or if the new method for acquiring TPPI data on a Varian system is used, a real FT should be performed along t_1, i.e., `proc1='rft'`. The command `wft2da`, which is equal to `wft2d('ptype',1,0,0,0,0,0,1,0)`, then properly processes 2D Bruker data.
- If the `'lp'` processing option is selected, additional parameters must be set to fully define how the time-domain data is to be manipulated. Refer to the description of the `addpar` or `parlp` macros in the *VNMR Command and Parameter Reference* for more information.

Occasionally, you may process a non-phase-sensitive data set in the phased (`ph`) mode by accident. Especially if pseudo-echo weighting has been used, the resulting data will not appear very pleasant! This problem cannot be cured if `pmode=''` without reprocessing the data. If `pmode='partial'`, enter the absolute-value mode command `av1` and redisplay the data with `dcon`. If `pmode='full'`, *any* mode of display along *both* frequency dimensions is fully accessible without having to reprocess the data.

Sign of f_1 Frequencies

Different experiments have the potential to produce sign reversals along f_1. Certain programs in the system, however, require that the sign of frequencies be the same in both dimensions. These include the axis labeling programs and the symmetrization program `foldt`. A simple method exists to overcome this problem without altering the experiment to be performed. Leaving the sign of the real part of a transform unchanged while reversing the sign of the imaginary part has exactly the effect of reversing the f_1 frequencies after transformation. Thus, multiplying all of the coefficients for the imaginary spectral part after the first transform by –1 will reverse the f_1 frequencies as desired. As an even simpler alternative, the keyword `'ptype'` may be supplied as an argument to the transform program such as `wft2d('ptype')`. In the absence of either the `'ntype'` or `'ptype'` keyword, `wft2d` defaults to the `'ntype'` option.

Once a particular set of coefficients is decided upon, a macro can be used to eliminate the necessity of typing four or eight coefficients each time. For example, if you perform the heteronuclear 2D-J experiment described above, you could create an appropriate macro.

wft2da is in fact a macro equivalent to wft2d(1,0,0,0,0,0,1,0) in some cases. Other macros for other combinations of coefficients can be created (see wft2da for an illustration).

2D Solvent Subtraction Filtering

In a 2D transform, solvent subtraction is invoked on t_2 FIDs in the same manner as 1D usage. The parameters ssfilter and ssorder select the processing option as follows:

- The zfs (zero-frequency suppression) option is selected if both ssfilter and ssorder are set to a value other than "Not Used."
- The lfs (low-frequency suppression) option is selected if ssfilter is set to a value other than "Not Used" and ssorder is set to "Not Used."
- The zfs and lfs options are both turned off if ssfilter is set to "Not Used."

These options are used with the ft1d, wft1d, ft2d, and wft2d commands, or with the ft1da, wft1da, ft2da, and wft2da macros.

Left Shift, Frequency Shift, Phase Rotation

If the parameter lsfid1 is set to a value other than 'n', the interferogram is left-shifted by lsfid1 complex (or hypercomplex) points before weighting and Fourier transformation are performed. The value of lsfid1 must lie between 0 and ni–1.

The parameter lsfrq sets a frequency shift of fn spectral data, in Hz, with a negative value resulting in peaks being shifted upfield (to the right) and a positive value in peaks being shifted downfield (to the left). lsfrq is the time-domain equivalent of lp within VNMR. lsfrq operates on complex np FID data, referred to as the t_2 dimension in a 2D experiment. Similarly, the parameters lsfrq1 and lsfrq2 set a frequency shift of the fn1 and fn2 spectrum, respectively.

If the parameter phfid1 is set to a value other than 'n', the interferogram is phase-rotated by phfid1 degrees (zero-order phase rotation) before weighting and Fourier transformation are performed.

If the parameter daslp exists, each interferogram is phase-rotated by daslp times (interferogram number) degrees before weighting and Fourier transformation are performed. This phase-rotation has the effect of "shearing" f1 traces of a 2D data set.

2D Processing of 3D Data

Acquisition and full processing of 3D data using VNMR is available provided the parameters ni2 and sw2 have been created (d3 is the incremented delay in the ni2 dimension). Also available is 2D processing of "slices" of the 3D data matrix, which can be performed as described below.

ft2d('ni2') transforms non-arrayed 2D data that have been collected with ni2 and sw2 (instead of ni and sw1). The addpar('3d') macro creates the necessary processing parameters for the ft2d('ni2') operation (par3d functions the same as addpar('3rf')).

ft2d('ni',#) is used to selectively transform a particular np–ni 2D plane within a non-arrayed 3D data set; # is an integer that can range from 1 to ni2 in this example.

ft2d('ni2',#) is used to selectively transform a particular np–ni2 2D plane within a non-arrayed 3D data set; # is an integer that can range from 1 to ni in this example.

If an arrayed 3D data set is to be selectively processed, the format of the arguments to `ft2d` changes. For example, `ft2d('ni',#1,#2)` performs a 2D transform along `np` and `ni` of the #2-th `ni2` increment and the #1-th element within the explicit array. This yields a 2D `np`–`ni` frequency plane. #1 ranges from `1` to `ni2`; #2 ranges from `1` to `[arraydim/(ni*ni2)]`.

Arrayed 3D data sets can also be subjected to 2D processing to yield 2D absorptive spectra. If the States-Haberkorn method is used along both f_1 (`ni2` dimension) and f_2 (`ni` dimension), there will generally be four spectra per (`ni`,`ni2`) 3D element. In this case, the command `ft2d('ni2',#1,<16 coefficients>)` would perform a 2D transform along `np` and `ni2` of the #1-th `ni` increment using the ensuing 16 coefficients to construct the 2D t_1-interferogram from appropriate combinations of the four spectra per (`ni`,`ni2`) 3D element. Use the `proc2` parameter to specify the type of data processing to be performed on the `ni2` interferogram (3D): `'ft'` for complex FT, `'rft'` for real FT, or `'lp'` for linear prediction processing on complex data. The macro `dg2` displays 3D processing parameters.

3.9 2D and 3D Linear Prediction

Just as in 1D linear prediction, the technique of linear prediction can be used in 2D and 3D data processing. Table 23 lists 2D and 3D linear prediction (LP) parameters and macros to create and display the parameters. For more information on the specific parameters, refer to the manual *VNMR Command and Parameter Reference*.

Table 23. 2D and 3D Linear Prediction (LP) Commands and Parameters

Commands	
`addpar('lp')`	Add LP parameters to current experiment
`dglp`	Display group of LP parameters
`parlp`	Create parameters for LP
Parameters	
`lpalg1` {'lpfft', 'lparfft'}	LP algorithm for `ni` dimension
`lpalg2` {'lpfft', 'lparfft'}	LP algorithm for `ni2` dimension
`lpext1` {number}	LP data extension for `ni` dimension
`lpext2` {number}	LP data extension for `ni2` dimension
`lpfilt1` {number}	LP coefficients to calculate, `ni` dimension
`lpfilt2` {number}	LP coefficients to calculate, `ni2` dimension
`lpnupts1` {number}	LP number of data points, `ni` dimension
`lpnupts2` {number}	LP number of data points, `ni2` dimension
`lpopt1` {'b', 'f'}	LP algorithm data extensions, `ni` dimension
`lpopt2` {'b', 'f'}	LP algorithm data extension, `ni2` dimension
`lpprint1` {number}	LP print output for `ni` dimension
`lpprint2` {number}	LP print output for `ni2` dimension
`lptrace1` {number}	LP output spectrum, `ni` dimension
`lptrace2` {number}	LP output spectrum, `ni2` dimension
`proc1` {'ft', 'rft', 'lp'}	Type of processing on `ni` interferogram
`proc2` {'ft', 'rft', 'lp'}	Type of processing on `ni2` interferogram
`strtext1` {1 to ni/2}	Starting point for LP data extension, `ni` dimension
`strtext2` {1 to ni2/2}	Starting point for LP data extension, `ni2` dimension
`strlp1` {number}	Starting point for LP calculation, `ni` dimension
`strlp2` {number}	Starting point for LP calculation, `ni2` dimension

Linear prediction parameters are created for the evolution axes by entering `addpar('lp',1)` to create parameters for the t_1 axis, or by `addpar('lp',2)` to create parameters for the t_2 axis (3D experiments only). Macros `parlp(1)` and `parlp(2)` function the same as `addpar('lp',1)` and `addpar('lp',2)`, respectively.

The `dglp` macro displays the linear prediction parameters for both (or all three) domains. The parameter `proc1` controls the transformation process along t_1, and `proc2` controls the transformation process along t_2. Using the same method of transformation is not necessary along two (or three axes). You might, for example, employ a backwards linear prediction in t_2 of a 2D experiment and a forwards linear prediction along t_1, or perhaps a simple Fourier transformation along t_2 and a backwards linear prediction along t_1.

3.10 Phasing the 2D Spectrum

Table 24 summarizes the commands and parameters discussed in this section.

Table 24. Commands and Parameters for Phasing the 2D Spectrum

Commands	
`ds<(index)>,ds<(options)>`	Display a spectrum
Parameters	
`lp1` {–3600 to +3600, in deg}	Left phase in 1st indirectly detected dimension
`pmode` {'', 'partial', 'full'}	Processing mode for 2D data
`rp1` {–360 to +360, in deg}	Zero-order phase in 1st indirectly detected dim.
`trace` {'f1', 'f2', 'f3'}	Mode for *n*-dimensional data display

The phase constants `lp1` and `rp1` control the phase correction along f_1 in phase-sensitive data. In most 2D experiments, these should be near zero, but because of finite pulse widths and delays present in the pulse sequence, they may be far from zero. If the pulse sequence properly compensates for these pulse widths and delays, it is possible to have zero `lp1` and `rp1`. Most of the setup macro set `lp1` and `rp1` to zero so that the first display will indicate the need (if any) for phase correction in f_1. The same techniques as used in 1D phasing are employed here, with a minor difference.

1. Enter **`f full`** to display the full data matrix in a full chart display.
2. To phase the 2D spectrum, use the horizontal cursor present in the interactive display to identify a peak toward the right-hand edge of the spectrum. Note the trace number indicated at the top of the display (you can “memorize” this by setting **`r1`** equal to its value.)
3. Select one or more other traces at f_1 values more toward the center and left parts of the spectrum. If there is a diagonal in the spectrum with large peaks, these will be the most sensitive with which to work. Use **`r2`**, **`r3`**, etc. to “memorize” these trace values. A minimum of two is needed, one at the far right and one at the far left.
4. Enter **`ds(r1)`**. Phase this spectrum as you would a 1D spectrum using the Phase button in the displayed menu. Click the mouse on the peak displayed near the right edge of the spectrum. Phase up this spectrum (thus setting `rp1`). Do not “click” in the left part of the spectrum at this time.
5. Enter **`ds(r2)`**. The second trace appears. Click the mouse near the right edge of the spectrum (to fix `rp1` at the previously determined value) and do not rephase. Move the mouse to the peak at the left, click and phase it (thus setting `lp1`).

6. Enter **ds(r1)** to recheck rp1. Repeat the process again if necessary.

In homonuclear correlation spectra (such as NOESY, TOCSY, and ROESY), use the diagonal peaks for phasing. It there are strong cross-peaks, you can phase an f_1 trace exactly like a 1D spectrum. Phase HMQC spectra by progressively working from right to left, with several peaks selected along the way to make sure that lp1 does not go through an extra revolution that would induce some baseline roll.

Corrections in f_2 phasing may be obvious in the 2D data when they are not in the first increment 1D spectrum. If pmode='full' before the 2D transform, f_2 phasing may be corrected without retransforming by setting trace='f2' and using the same approach as described for f_1 phasing. Transformation of the data again is necessary if pmode='' or pmode='partial'. No f_1 phasing is possible after transformation if pmode=''; f_1 rephasing after the transform is possible (but not f_2 rephasing) if pmode='partial'. Do baseline corrections such as dc2d or bc only after data are properly phased in f_1 and f_2.

3.11 Display and Plotting

This section discusses noninteractive 2D display and plotting. Table 25 lists the many commands and parameters available. Interactive 2D color map display (the dconi program) and interactive 2D peak picking (the ll2d program) are covered on page 115 and page 120, respectively.

Display Modes

The commands for 2D display modes are analogous to the command and modes in f_2:

- ph1 selects the phase-sensitive mode in f_1. This is equivalent to the ph mode in f_2.
- pa1 selects the phase angle mode in f_1. This is equivalent to the pa mode in f_2.
- av1 selects the absolute-value mode in f_1. This is equivalent to the av mode in f_2.
- pwr1 selects the power mode in f_1. This is equivalent to the pwr mode in f_2.

The dmg1 parameter stores the display mode ('ph1', 'pa1','av1', or 'pwr1') for f_1. It can be set with the commands ph1, pa, av1, and pwr1, respectively. If dmg1 is not set to one of these values, the display mode for f_1 is then selected based upon dmg, the display mode parameter both for f_2 in 2D data and for 1D data.

Display and Plot Limits

The center, left, right, and full commands set the parameters sc, wc, sc2, and wc2 as appropriate to produce a display (and subsequent plot) in the relevant portion of the screen (and page) described by the command. The parameter sc2 is the start of the chart in the second direction and the parameter wc2 is the width of the chart. Together, sc2 and wc2 control the dimensions of the second axis (or the y axis) in a 2D contour plot.

The asize macro adjusts the parameters sc, wc, wc2, and sc2 so that the displayed resolution along both f_1 and f_2 is the same. The use of asize is not suggested for heteronuclear experiments where the chemical shift spread of one nucleus is much greater than that of the other.

Another command, fullt, sets display limits for a full screen with room for traces. The parameter sp1 is the start of the plot in f_1, and wp1 is the width of the plot. Both are analogous to the corresponding sp and wp 1D parameters.

Table 25. 2D Display and Plotting Commands and Parameters (Part 1 of 2)

Command	
acosy	Automatic analysis of COSY data
acosyold	Automatic analysis of COSY data, old algorithm
asize	Make plot resolution along f_1 and f_2 the same
av1	Select AV mode along 1st indirectly detected dimension
center	Set display limits for center of screen
centersw1	Move cursor to center of spectrum in 1st indirect dimension
crl1	Clear reference line in 1st indirectly detected dimension
dcon<(options)>	Display noninteractive color intensity map
df2d<(array_index)>	Display FIDs of 2D experiment
dpcon*	Display plotted contours
dpconn*	Display plotted contours without erasing screen
ds<(index)>, ds<(options)>	Display a spectrum
ds2d<(options)>	Display 2D spectra in whitewash mode
ds2dn<(options)>	Display 2D spectra in whitewash mode without erasing
dss*	Display stacked spectra
dsww*	Display spectra in whitewash mode
foldcc	Fold INADEQUATE data about 2-quantum axis
foldj	Fold J-resolved 2D spectrum about f1=0 axis
foldt	Fold COSY-like spectrum along diagonal axis
full	Set display limits for a full screen
fullt	Set display limits for a full screen with room for traces
grid<(<spacing,><colors>)>	Draw a grid on a 2D display
image	Display noninteractive gray scale image
imageprint	Plot noninteractive gray scale image
left	Set display limits to left half of screen
nm2d<(noise_mult)>	Normalize intensity of 2D spectrum
noisemult<(noise_mult)>	Control noise multiplier for automatic 2D processing
pa1	Set phase angle mode in 1st indirectly detected dimension (C)
pa	Set phase angle mode in indirectly detected dimension (C)
pacosy	Plot automatic COSY analysis
pcon*	Plot contours on a plotter
peak2d*	Return information about maximum in 2D data
ph1	Select phased mode on 1st indirectly detected dimension
pl*	Plot spectra
pl2d*	Plot 2D spectra in whitewash mode
plgrid*	Plot a grid on a 2D plot
plww*	Plot spectra in whitewash mode

```
* dpcon(<options,><levels,spacing>)
  dpconn(<options,><levels,spacing>)
  dss<(<start,finish<,step>><,options>)>
  dsww<(<start,finish<,step>><,'int'>)>
  pcon<(<'pos'|'neg'><,'noaxis'><,levels><,spacing>)>
  peak2d:$maximum_intensity<,$trace,$point>
  pl<(<start,finish<,step>><,'int'><,'all'><,options>)>
  pl2d*<('nobase'|'fill'|'fillnb')>
  plgrid<(<spacing><,><pen>)>
  plgrid<(start_f2,increment_f2,start_f1,increment_f1<,pen>)>
  plww<(start,finish,step><,'all'>)>
```

Continued on next page

Table 26. 2D Display and Plotting Commands and Parameters (Part 2 of 2)

Command	
`proj*`	Project 2D data
`pwr1`	Select power mode on 1st indirectly detected dimension
`right`	Set display limits to right half of screen
`rl1<(frequency)>`	Set reference line in 1st indirectly detected dimension
`rotate<(number_degrees)>`	Rotate 2D data
`setsw1*`	Set spectral width in 1st indirectly detected dimension
`* proj(exp_number<,'sum'><,start<,width>>)`	
`setsw1(nucleus,downfieldppm,upfieldppm):offset`	
Parameter	
`axis*`	Axis label for displays and plots
`cr1` {frequency, in Hz}	Cursor position in 1st indirectly detected dimension
`dmg` {'pa','ph','av','pwr'}	Display mode for data
`dmg1` {'pa1','ph1','av1','pwr1'}	Display mode along 1st indirectly detected dimension
`sc` {0 to wcmax, in mm}	Start of chart
`sf` {0 to the value of at, in sec}	Start of FID
`sf1` {0 to (2xni)/sw1, in sec}	Start of interferogram in 1st indirectly detected dimension
`sc2` {0 to wc2max, in mm}	Start of chart in second direction
`sp` {number, in Hz}	Start of plot in directly detected dimension
`sp1` {number, in Hz}	Start of plot in 1st indirectly detected dimension
`scalesw1` {'n', number > 0.0}	Scale spectra width in 1st indirectly detected dimension
`trace` {'f1','f2','f3'}	Mode for n-dimensional data display
`vs2d`	Vertical scale for 2D displays
`wc` {5 to wcmax, in mm}	Width of chart
`wc2` {number, in mm}	Width of chart in second direction
`wf` {0 to the value of at, in sec}	Width of FID
`wf1` {0 to (2xni)/sw1, in sec}	Width of interferogram in 1st indirectly detected dimension
`wp`{number, in Hz}	Width of plot in directly detected dimension
`wp1` {number, in Hz}	Width of plot in 1st indirectly detected dimension
* `axis` {'1','2','3','c','d','h','k','m','n','p','u'}	

Maximum Intensity

The `peak2d` command searches the area defined by `sp`, `wp`, `sp1`, and `wp1` in a 2D data set for a maximum intensity. It returns the maximum intensity value found, the trace number of the maximum, and the data point number of the maximum on that trace.

Axis Label and Direction

For 2D, the parameter `axis` has two letters, with the first letter describing the detected spectral axis (f_2), and the second letter describing the indirectly detected axis (f_1). The special letter `d` is added to reference any indirectly detected axis to the parts per million of the decoupler channel. The special letter `e` is added to reference any indirectly detected axis to the parts per million of the second decoupler channel. The letter `n` is used to suppress the axis display on one or both axes.

The parameter `trace` selects the horizontal axis. `trace='f1'` displays f_1 axis horizontally so that f_1 traces can be displayed. `trace='f2'` displays f_2 axis horizontally so that f_2 traces can be displayed.

Display Scaling

The `nm2d<(noise_mult)>` macro sets up the parameters `vs2d` and `th` automatically for a 2D contour plot and color map display. `nm2d` measures the highest signal in the spectrum and sets `vs2d` such that the highest signal is in the range of the highest color level. It then measures the root-mean-square noise in the second trace, both in f_1 and f_2 (the f_1 trace may contain an f_1 axial signal if FAD was applied). From both traces, `nm2d` measures two regions, avoiding the center and both ends of the trace. From the four resulting root-mean-square noise figures, the lowest value is taken and multiplied with a noise multiplier, either the value specified by the argument `noise_mult` or the default (8 for ^{1}H, ^{19}F and ^{31}P [high-dynamic-range nuclei] and homonuclear spectra in general, or 3 for other spectra) if no argument is present. If the multiplied noise figure is below `th=1`, `vs2d` is scaled up; otherwise, `th` is increased to the desired level. `nm2d` works both with absolute-value and phase-sensitive spectra. `trace` can be set to `'f1'` or `'f2'`.

The macro `noisemult<(noise_mult)>` predetermines the noise multiplier used by `nm2d` when starting automatic 2D experiments. The default is 8 for homonuclear 2D spectra or 4 for other spectra. The argument `noise_mult` overrides the default.

Grid Lines

A grid of horizontal and vertical lines over a 2D display can be drawn by the `grid` macro. By default, grid lines are drawn in blue at approximately 1 cm intervals, rounded so that the intervals fall at a multiple of 1, 2, or 5 of Hz or ppm. To change the defaults, enter `grid` with a different spacing (in cm) or a different color (`'red'`, `'green'`, etc.); for example, `grid(2,'white')` gives white grid lines at 2 cm intervals.

The `grid` command also can define a grid, using the following syntax:
`grid<(startf2,incrf2,startf1,incrf1,color)>`

The arguments define the frequency and increments between grid lines in the f_2 and f_1 directions and the color of the grid lines.

The `plgrid` macro uses the same arguments as `grid`, but plots the `grid` instead.

Color Maps and Contour Plots

The main command for 2D color map display is `dconi`, but it is interactive and described separately on page 115. The rest of the 2D display commands are noninteractive.

- `dcon` produces a “contour plot” (actually a color intensity map) on the screen. `dconn` performs the same as `dcon` except `dconn` does not erase the screen before starting the display. Both `dcon` and `dconn` accept various options as arguments.
- `image` macro displays a `dcon` noninteractive display of an image using grayscale and linear scaling of the intensity. This macro is useful for adjusting the display while using the `dconi` command.The `imageprint` macro can send the same image to the plotter.
- `dpcon(<mode,><levels,spacing>)` produces a true contour plot display, and `pcon<(mode,><levels,spacing)>` produces a contour plot on the plotter. `levels` represents the maximum number of contour levels that will be shown; the default is 4. `spacing` represents the spacing of successive contour levels; the default is 2. `dpconn(<mode,><levels,spacing>)` produces the same display as `dpcon` but does not erase the screen before starting. In phase-sensitive spectra, `mode` can be `'pos'`, to display positive peaks only, or `'neg'`, to display negative peaks only.

- `df2d<(array_index)>` produces a color intensity map of the raw 2D FIDs as a function of t_1 and t_2, where `array_index` is the index of the array to display. The display can be modified by subsequent display commands.

Whitewashed Spectra

The `dsww<(start,finish,step)>` command displays one or more spectra with whitewashing (traces in front "block" the view of traces behind them). Use the argument `'all'` to display all spectra. `plww<(start,finish,step)>` plots the same spectra.

The `ds2d<(options)>` command produces a stacked display of 2D spectra in the whitewash mode. Certain options are available: `'nobase'` activates the `th` parameter to suppress all intensity below `th`, `'fill'` fills in the peaks, and `'fillnb'` combines base suppression and peak filling. The `ds2dn<(options)>` command creates the same stacked display as `ds2d` but does not erase the screen before starting. A stacked plot is produced by `pl2d<(options)>`.

Equivalent nonwhitewashed stacked displays and plots may be obtained with `dss` and `pl`, respectively.

Label Display

The `dssl` macro displays a label for each element in a set of stacked spectra. The label is an integer value starting with 1 and ranging to the number of spectra in the display.

If `wysiwyg='n'`, labels can appear at incorrect positions. The positions were empirically determined for a large screen display and are not guaranteed to be correct for all displays.

The following `options` control the `dssl` display (more than one option can be entered as long as the options do not conflict with each other):

- `'center'`, `'left'`, `'right'`, `'top'`, `'bottom'`, `'above'`, and `'below'` are keywords setting the position of the displayed index relative to each spectrum.
- `'value'` is a keyword that produces a display of the values of each array element, instead of an integer index.
- `'list=xxx'` produces a display of the values contained in the arrayed parameter `xxx`.
- `'format=yyy'` uses the format `yyy` to control the display of each label. See the `write` command for information about formats.

Projection of 2D Data

The `proj(exp_number<,'sum',start,width>)` command projects data onto the axis parallel to the screen *x*-axis, which can be either the f_1 or f_2 axis, depending upon the parameter `trace`

- In a skyline projection, the data is searched and the maximum intensity at any given frequency becomes the intensity in the projection.
- In a summing projection, the data at each frequency are summed and the result becomes the projection. `start` and `width` (in Hz) define the traces to be projected.
- If omitted, the whole data set is projected.

The argument `exp_number` is the number of the experiment in which the resulting spectrum is stored. If the `'sum'` keyword is given, the projection is calculated as the sum of the data; otherwise, as the maximum (skyline projection).

2D Referencing

The macros `rl` and `rl1` set the direct dimension and first indirectly detected dimension reference lines, respectively. By default, each reference line is set at the cursor position (`cr` for direct dimension and `cr1` for the first indirect dimension) after taking into account any frequency scaling with the `scalesw` or `scalesw1` parameters, respectively.

To set the reference lines to other than the cursor position, include the `frequency` argument. A number for `frequency` with no suffix (e.g., `rl1(0)`) indicates the frequency in hertz. A number with the suffix `p`, `d`, or `k` (e.g., `rl1(7.2p)`) indicates the frequency in ppm, decoupler ppm, or kilo, respectively. These suffixes are defined by the unit command. The default definition is multiplication by (respectively) `reffrq`, `dfrq`, and 1000. Thus, if you are doing a 2D experiment in which the indirect axis is determined by the decoupler channel (e.g., HMQC or HETCOR experiment), you might enter, for example, `rl1(10d)`, which is equivalent to `rl1(10*dfrq)`. The `refsource1` parameter is used to signify whether the frequency along the indirect dimension is related to the observe transmitter (`refsource1='sfrq'`) or if it is related to the decouple frequency (`refsource1='dfrq'`).

The command `crl` sets the reference parameters `rfl` and `rfp` to zero and sets `refpos1='n'`. In 2D spectra, this clears the referencing along f_2. In 2D spectra the command `crl1` can be used to set `rfl1` and `rfp1` to zero and `refpos1='n'`, therefore clearing referencing along f_1.

The macro `setsw1(nucleus,downfieldppm,upfieldppm)` sets the spectral width parameter `sw1` for a given spectral window. `setsw1` also does referencing.

The macros `centersw` and `centersw1` set the cursor to the center of the spectrum in the directly detected dimension and the first indirect dimension, respectively.

The macros `setref1` and `setref2` set frequency referencing using the `setref` macro. Given a nucleus (e.g., `tn` or `'C13'`), `setref1(nucleus)` calculates the value of `rfl1`, `rfp1`, `refpos1`, and `reffrq1`; and `setref2(nucleus)` calculates `rfl2`, `rfp2`, `refpos2`, and `reffrq2`.

Rotating Homonuclear 2D-J Spectra

The `rotate<(angle)>` command rotates homonuclear 2D-J data 45° (rotation in frequency-space) to line up multiplets. Use the `angle` argument to specify other angles.

Symmetrizing Data

The `foldt`, `foldj`, and `foldcc` commands symmetrize data as follows:

- `foldt` symmetrizes or triangularizes COSY, NOESY, or similar 2D spectra, by “folding” about the diagonal; it requires `fn=fn2` and `sw=sw1`.
- `foldj` symmetrizes heteronuclear 2D-J or rotated homonuclear 2D-J experiments by “folding” along *f1*=0 (*J*=0) axis.
- `foldcc` symmetrizes 2D INADEQUATE data along the appropriate axis and also applies an automatic dc correction.

Setting Negative Intensities to Zero

The command `zeroneg` is used for the projection of proton 2D-J spectra at 45° to strip a high resolution proton spectrum down to a list of chemical shifts. `zeroneg` sets all negative intensities to zero.

Automatic Analysis

The `acosy` and `acosyold` commands automatically analyze a COSY data set with `fn=fn1` and `sw=sw1` (`acosyold` uses an older algorithm from previous VNMR software versions). Symmetrization of the data with the command `foldt` is recommended, but not required.

1. Select a proper threshold and perform a 2D line listing with the command `ll2d('peak','volume')`.
2. Display the 2D data with the command `dcon`, leaving enough room at the left side of the display for the connectivity table.
3. Enter `acosy` or `acosyold` to analyze the data and display the connectivities on the screen.

The command `pacosy` performs the same analysis as `acosy` and plots the connectivities on a plotter.

3.12 Interactive 2D Color Map Display

The `dconi<(options)>` command interactively displays 2D traces and projections, which can also be plotted. The following keywords can be used as optional arguments:

- `'again'` means identify current screen mode and redraw the screen in that mode.
- `'avcolor'` means use absolute-value color set and display positive peaks only.
- `'dpcon'` means display true contour plot.
- `'ds2d'` means display “whitewashed” stacked plot.
- `'gray'` means use grayscale color set.
- `'linear'` means display in linear instead of logarithmic increments.
- `'phcolor'` means use phased color set, and display positive and negative peaks.
- `'restart'` means activate `dconi` without redrawing the 2D data set (make sure that 2D data is already displayed).

When `dconi` in invoked with the `'dpcon'` keyword, it draws the 2D data on the screen similar to Figure 24.

At the top of the display (directly below the Permanent menu) is the Main menu for `dconi`. Below the menu is a graphics window with a 2D display box and, if the `'dcon'` option is selected, a color/grayscale adjustment bar to the right of the box. The default display mode is the color map drawn by `dcon`, but optional keywords `'dpcon'` and `'ds2d'` can be used to override the default.

Readouts for parameters such as the following appear in the lower part of the screen:

- `cr` shows the current cursor position.
- `cr1` shows the current cursor position along the first indirectly detected dimension.
- `delta` shows the cursor difference.
- `delta1` shows the cursor difference along the first indirectly detected dimension.
- `vs2d` shows the vertical scale of the display.
- `vsproj` shows the vertical scale of the trace or projection.

Table 27 lists commands and parameters associated with the `dconi` program.

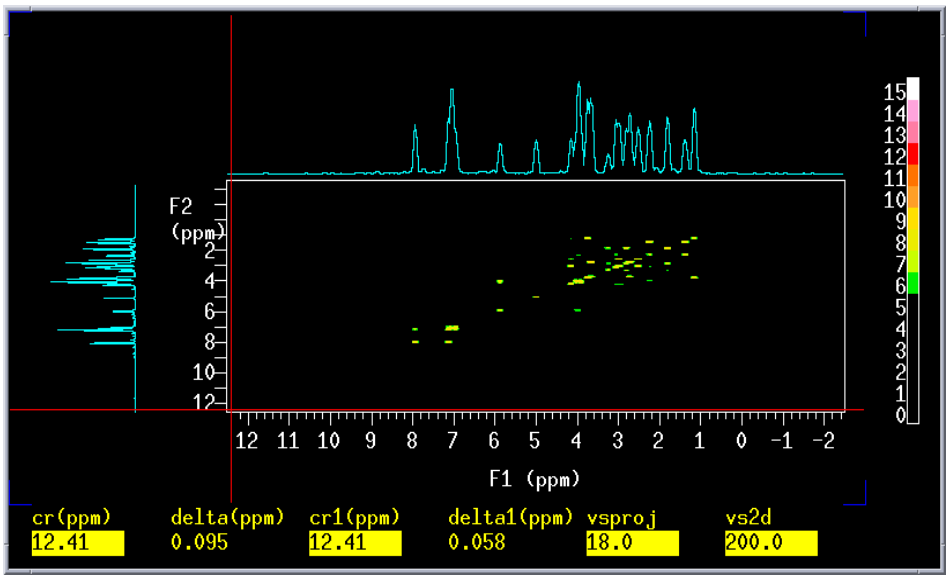


Figure 24. Interactive 2D Contour Display (dconi Program)

Table 27. Interactive 2D Color Map Display Commands and Parameters

Commands	
`boxes<('graphics'|'plotter')>`	Draw boxes selected by the `mark` command
`dconi<(options)>`	Interactive 2D contour display
`vsadj`	Automatic vertical scale adjustment
Parameters	
`cr` {number}	Current cursor position
`cr1` {number}	Cursor position along 1st indirectly detected dimension
`dconi`*	Control display selection for the dconi program
`delta` {pos. number, in Hz}	Difference of two frequency cursors
`delta1` {pos. number, in Hz}	Cursor difference in 1st indirectly detected dimension
`grayctr` {0 to 64}	Gray level window adjustment
`graysl` {–10 to +10}	Gray level slope adjustment
`sp` {number, in Hz}	Start of plot in directly detected dimension
`sp1` {number, in Hz}	Start of plot in 1st indirectly detected dimension
`vs2d` {1e–6 to 1e9}	Vertical scale for 2D displays
`vsproj`	Vertical scale for projections and traces
`wp` {number, in Hz}	Width of plot
`wp1` {number, in Hz}	Width of plot in 1st indirectly detected dimension
* `dconi` {'','display_program',' display_program,option1,option2'}	

The string parameter `dconi` also controls the display selection. For example, if the parameter `dconi` is set equal to the string `'dpcon,pos,12,1.2'`, the `dconi` command displays twelve positive contours with `dpcon`, using a spacing of 1.2. For details on using the `dconi` parameter, refer to the *VNMR Command and Parameter Reference*.

Interactive 2D Display Menus

Upon starting the `dconi` program, the 2D Display Main Menu is active with the following buttons:

By clicking on the Proj button, the following 2D Display Projection Menu replaces the 2D Display Main Menu:

Each of the buttons on these menus is described in the sections below.

Interactive 2D Color Map Display Main Menu

The buttons the Interactive 2D Color Map Display Main Menu function as follows:

Box	The first button is labeled Box or Cursor, depending on the display mode you are in. If labeled Box, you are in the cursor mode, and this button changes the display to he box mode with two pairs of cursors.
Cursor	If labeled Cursor, you are in the box mode, and this button changes the display to the cursor mode with one pair of cursors.
Trace	Selects the trace display mode.
Proj	Displays the Interactive 2D Display Projection Menu, see below.
Expand	The fourth button is labeled Expand or Full depending on the mode you are in. If labeled Expand, you are in the box mode and this button expands the area between the cursors.
Full	If labeled Full, you are in the cursor mode and this button displays the full area.
Redraw	Repeats the last 2D or image display with current parameters.
Plot	Plots the current trace.
Peak	Displays the Interactive 2D Peak Picking Main Menu—`ll2d` program (see page 123).
Return	Returns to the previous menu.

Interactive 2D Display Projection Menu

The buttons the 2D Display Projection Menu function as follows:

Hproj (max)	Displays a horizontal projection of the maximum intensity at each frequency.
Hproj (sum)	Displays a horizontal projection of the summed intensity at each frequency.
Vproj (max)	Displays a vertical projection of the maximum intensity at each frequency.
Vproj (sum)	Displays a vertical projection of the summed intensity at each frequency.
Plot	Plots the current projection.
Cancel	Returns to the Interactive 2D Color Map Display Main Menu, see above.

Controlling the Display with the Mouse

The left and right mouse buttons are used to move cursors, the center button to adjust the vertical scale of traces, projections and contour maps, as well as to adjust the threshold in the color bar. The cursors can be used to select regions for expansions of the display. The cursors can also be used to select positions to “mark” using either the `mark` command or the `ll2d('mark')` command. Both commands display and record spectral frequencies, maxima, intensities, and volumes. `ll2d('mark')` is recommended, however, because it also allows for interactive display and editing of mark locations.

Left Mouse Button

The left mouse button adjusts the position of the 2D cursor. The corresponding frequencies are displayed at the bottom of the graphics window. Both the horizontal and vertical cursors move if the left mouse button is pressed within the 2D display box.

Above and below the box, only the vertical cursor can be moved; at the left and the right of the box, only the horizontal cursor. In addition, holding the mouse button down and then moving the mouse moves the cursor with the mouse. This “dragging” mode is not available on the GraphOn terminal for speed reasons.

Center Mouse Button

The function of the center mouse button depends on the location of the cursor:

- If the cursor is within the 2D display box, in gray scale images, pressing the center button sets the point to medium gray. Otherwise, for color map and contour displays, if there is no intensity at that point, the center button changes vertical scale to show intensity at that point. If there is intensity at the point, the center button changes the scale to show no intensity, then changes the parameter `vs` and redraws.
- If the cursor is near an active trace and active horizontal or vertical projection, pressing the center button changes the vertical scale of trace or projection, so that spectrum goes through the current mouse position.
- If the cursor is near the color/grayscale bar and in the color mode, pressing the center button sets the threshold to remove low intensity peaks. If in the grayscale mode, pressing the center button sets the grayscale intensity (the right button adjusts contrast).

Right Mouse Button

A second cursor pair is displayed with the right mouse button. The second pair can be moved in exactly the same way as the first pair, and is used to select a box within the 2D display. The right mouse button also switches the display into the box mode, the same as clicking on the Box button in the menu.

Changing the Display

The user interactively modifies the display through selecting buttons on the menus, pressing the buttons on the mouse, and moving the mouse. If desired, commands and macros can also be typed in at any time.

Displaying Traces

The Trace button can be used to display a trace at the current position of the first horizontal cursor. The left mouse button still moves the cursor, and different traces will be selected and displayed accordingly.

The vertical scale of the trace can be adjusted with the center mouse button, by placing the mouse arrow above the spectrum at the requested height and then pressing this button. Do not press the center button at this time within the 2D display box.

The trace mode can be left by displaying a box with the right mouse button, or by selecting any other display mode.

Displaying Projections

With the Proj button, you can display projections from the 2D Display Projection Menu. Projections can be made in horizontal and vertical direction, and are available as the sum or maximum of the data. Select one of the four available modes or cancel the operation with the Cancel button. Again, the center mouse button adjusts vertical scales.

Expanding the Display

Once a box is selected, you can click on the Expand button to obtain an expanded display. Alternatively, if you are in the one cursor mode, you can click on the Full button to display the full 2D display. In this case, the two cursor pairs mark the last expanded region and the Full/Expand button toggles between the full and expanded mode.

An alternative way to select an expansion is to type in new values for the parameters `sp`, `wp`, `sp1`, and `wp1` (e.g., `sp=100 wp=50 sp1=100 wp1=50)`, then use the Redraw button to redisplay.

Setting the Vertical Scale

The vertical scale can be adjusted in a number of ways. If a peak is expected at a certain position in the spectrum but is not visible, the mouse arrow can be moved to that position and then the *center* mouse button pressed once. This selects a new vertical scale, so that the intensity at that point is by a factor of 2 above the threshold, and the display is redrawn. Be careful in this mode not to queue up several redraw operations.

Adjusting the Threshold

If noise is visible at a certain position in a spectrum, but should be suppressed below the threshold, move the mouse arrow to that position and press the center button. A vertical scale is calculated so that this intensity falls by a factor of 2 below the threshold, and again the spectrum is redrawn. If the peak is visible but is not a factor of 2 above the threshold, clicking on the center button increases `vs2d`.

On Sun color screens only, the threshold of the 2D display can be adjusted in real time. For color displays, the threshold is adjusted by placing the mouse button on the color bar at the right edge of the display, selecting one of the colors and pressing the center mouse button. All colors below that level are set to black. For grayscale images on the Sun color display, the center of the grayscale is adjusted in the same way. At the same time, the grayscale is expanded by a factor of 4. On monochrome terminals, this mode is not available.

In order to perform threshold adjustment on grayscale images, two new parameters must be created: `grayctr`, which controls the center of the grayscale, and `graysl`, which

controls the slope of the grayscale. Enter the `parim` macro to create `grayctr` and `grays1`, or create the parameters by hand as follows:

- To create `grayctr`, enter
```
create('grayctr','real')
setgroup('grayctr','display')
setlimit('grayctr',64,0,1).
```
- To create `graysl`, enter
```
create('graysl','real')
setgroup('graysl','display')
setlimit('graysl',10,-10,0.01).
```

If these parameters do not exist, the interactive display still lets you adjust the grayscale threshold and contrast, but these adjustments are not retained.

Treating 2D Traces as 1D Spectra

After a trace has been selected in the interactive 2D display program, entering the command `ds` allows the trace to be displayed as if it were a simple 1D spectrum. All standard 1D data manipulations, including line listing, integration, etc., are then accessible for that trace. The command `ds(tracenumber)` also can be used to display an f_1 or f_2 trace, depending on the value of `trace`.

3.13 Interactive 2D Peak Picking

The `ll2d` program is used to automatically or interactively pick peaks in 2D spectra or 2D planes of 3D spectra. The peaks can be displayed on top of the spectrum in the `dconi` display or can be plotted using the `pll2d` command. Table 28 lists commands and parameters related to the `ll2d` program.

Table 28. Interactive 2D Peak Picking Commands and Parameters

Commands	
`addpar('ll2d')`	Add ll2d parameters to the current experiment
`dconi<(options)>`	Interactive 2D contour display
`ll2d*`	Automatic and interactive 2D peak picking
`ll2dbackup<(file)>`	Copy current ll2d peak file to another file
`parll2d`	Create parameters for 2D peak picking
`pll2d<(options)>`	Plot results of 2D peak picking
`* ll2d<(options)><:$num>, ll2d('info'<,#>):$peak_number,$f1,$f2,$amplitude,$volume,$label, $comment,$FWHH1,$FWHH2,$f1_min,$f1_max,$f2_min,$f2_max`	
Parameters	
`ins` {number}	2D volume value
`ins2ref` {number}	Fourier number scaled volume of a peak
`ll2dmode*`	Control display of peaks picked by `ll2d` program
`th2d` {0.0 to 1.0]	Threshold for integrating peaks in 2D spectra
`xdiag` {number, in Hz}	Threshold for excluding diagonal peaks when peak picking
* `ll2dmode` {4 characters from 'y' and 'n'}	

The results of all peak picking operations are stored in a binary file in the `ll2d` subdirectory of the current experiment directory:

- For 2D spectra, the results are stored in the file `peaks.bin`.

- For 2D planes of 3D spectra, the results are stored in `peaks_f#f#_#.bin`, where `f#f#` denotes the orientation of the plane being picked (e.g., `f1f3` or `f2f3`) and the last # denotes the number of the plane.

Binary peak files can be converted to text files for printing or for export to other programs.

For each peak in a peak file, the following information is stored:

- Peak number
- Interpolated peak frequency in both dimensions
- Interpolated peak amplitude
- Full width at half-height (FWHH) in both dimensions
- Bounds of the peak in both dimension
- Volume of the peak
- 15-character peak label
- 80-character comment

The parameter `ins2` adjusts the 2D volume value. Volume is independent of `is` and `vs2d`. It is scaled by Fourier numbers for the two dimensions.

The parameter `ins2ref` is set to the Fourier number scaled volume of the selected peak. The reported volume is *volume*`*ins2/ins2ref/fn/fn1`. If `ins2ref` is "not used," the sum of all volumes is `ins2`. The "not used" mode is equivalent to a "normalized" volume mode. If `ins2ref` is zero or not defined, the reported volumes will be *volume*`*ns2/fn/fn1`.

A typical use of `ins2ref` would be to position a cursor within a peak region and set `ins2ref` equal to the scaled volume returned by the command `ll2('info')`. The reported volume of that peak would then be the value of `ins2`. This operation is analogous to the 1D integral scheme.

- `vs2d` shows the vertical scale of the display.
- `vsproj` shows the vertical scale of the projection or trace.

The options listed below are available for `ll2d`:

- `'peak'` is a keyword to find all peaks above the current threshold in the area of the spectrum displayed in `dconi` (if in cursor mode) or the area defined by the cursors (in box mode). This option gives each peak a number and determines peak frequencies and amplitude.
- `'volume'` is a keyword that, for all peaks picked using the `'peak'` option, finds the bounds, volume, and FWHH) of the peak in both dimensions.
- `'adjust'` is a keyword to adjust the bounds of all peaks in the displayed area so that none overlap, and then to recalculate volumes.
- `'reset'` is a keyword to delete all peaks in the spectrum.
- `'read'` is a keyword to prompt for a binary peak file name and read in that file.
- `'read',file` reads in a binary peak file named file.
- `'readtext'` is a keyword to prompt for a text peak file name and read in that file.
- `'readtext',file` reads in a text peak file named file.
- `'writetext'` is a keyword to prompt for a file name for a peak file and write out a text file with that filename.
- `'writetext',file` writes a peak file to a text file with the name given by file.
- `'draw'` is a keyword to draw peaks in the peak file to the graphics window.

- `'mark'` is a keyword to insert a peak at the current cursor location (in the `dconi` cursor mode) or to use the area defined by the cursors as peak bounds and calculate the volume in this area (in the `dconi` box mode). This option assigns these bounds to each peak within this area that does not have its bounds already defined. If a peak without bounds defined does not exist in this area, it finds the highest point in this area, marks it as a peak, and assigns it the bounds defined by the cursors.
- `'unmark'` is a keyword to delete the peak nearest the cursor (in `dconi` cursor mode) or to delete all peak bounds which are completely within the area defined by the cursors (in box mode). The peaks are not deleted in box mode.
- `'unmark',#` deletes peak number #.
- `'clear'` is a keyword to delete all peaks within the displayed area (in `dconi` cursor mode) or to delete all peaks within the area defined by the cursors (in box mode).
- `'label'` is a keyword to prompt for a 15-character label. The label is assigned to the nearest peak (`dconi` cursor mode) or to all peaks within the area defined by the cursors (`dconi` box mode).
- `'label',string` executes the `'label'` option using the string argument instead of prompting for a label.
- `'label',string,#` assigns string to be the label of peak number #.
- `'comment'` is a keyword to prompt for a 80-character comment. The comment will be assigned to the nearest peak (cursor mode) or to all peaks within the area defined by the cursors (box mode).
- `'comment',string` executes the `'comment'` option using the string argument instead of prompting for a comment.
- `'comment',string,#` assigns string to be the comment of peak number #.
- `'info'` is a keyword to print information to the text window about the peak nearest the cursor.
- `'info','total'` prints the total number of peaks in the spectrum, or if a return value is requested, returns the total number of peaks in the spectrum.
- `'info',#` prints information to the text window about peak number `#.` If return values are requested, then printing is suppressed and the values are returned in this order: `peak_number`, `f1`, `f2`, `amplitude`, `volume`, `label`, `comment`, `FWHH1`, `FWHH2`, `f1_min`, `f1_max`, `f2_min`, `f2_max`.
- `'combine'` is a keyword to combine all peaks within the area defined by the cursors into a single peak (in `dconi` box mode only). The individual peaks to be combined are permanently deleted. You may wish to back up the peak file before using this option, since it is not possible to undo combining peaks.
- `'combine',#1,#2,...` performs the `'combine'` option on the list of peaks with numbers `#1`, `#2`,.... If a return value is requested, the value returned is the peak number of the new combination peak.
- `'pos'` or `'neg'` can be used in addition to `'peak'`, `'volume'`, or `'clear'` to operate only on either positive or negative peaks.

Display of peaks in `dconi` is controlled by the global parameter `ll2dmode`. This parameter has four characters, each of which can take the value `'y'` or `'n'`. The first character controls display of a "+" to mark the peak maximum, the second controls display of the peak number, the third controls display of the peak bounds, and the fourth controls display of the peak label.

The parameter `th2d` controls the threshold for integrating peaks, and the parameter `xdiag` excludes diagonal peaks within `xdiag` Hz of the diagonal from peak picking. If

these parameters do not exist, a default value is used for each. To use different values than the defaults, create both parameters by entering `addpar('ll2d')` and then setting the values as usual (the macro `parll2d` functions the same as `addpar('ll2d')`).

Interactive 2D Peak Picking Menus

Most of the above options are accessible through a series of user-programmable menus of the `dconi` program (described in "Interactive 2D Color Map Display," page 115). From the 2D Display Main Menu of `dconi`, the Peak button brings up the 2D Peak Picking Main Menu of the `ll2d` program with the following buttons:

These buttons provide access to the following menus (the labels on some buttons change depending on what mode you are in):

- 2D Peak Picking Automatic Menu for automatically picking peaks, selected by the Auto button in the 2D Peak Picking Main Menu (Figure 25 shows this menu with a typical `ll2d` screen):

- 2D Peak Picking Edit Menu for interactively editing peaks, selected by the Edit button in the 2D Peak Picking Main Menu:

- 2D Peak Picking File Menu for manipulating peak files, selected by the File button in the 2D Peak Picking Main Menu:

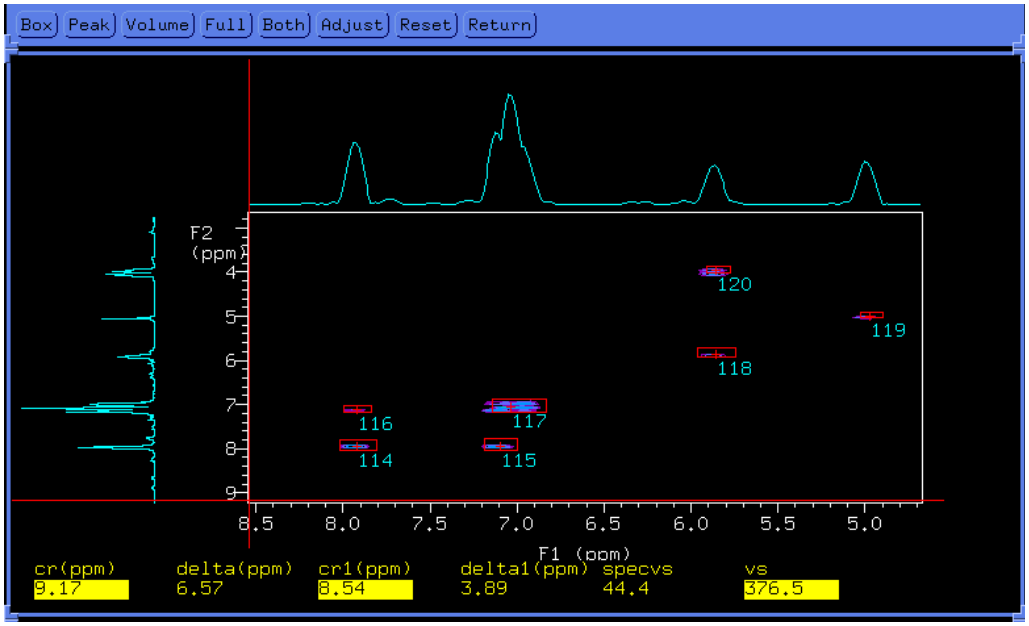


Figure 25. Interactive 2D Peak Picking (ll2d Program)

- 2D Peak Picking Display Menu for controlling the display of peaks, selected by the Display button in the 2D Peak Picking Main Menu.

Each of the buttons on these menus is described in the sections below.

2D Peak Picking Main Menu

This menu selects another 2D peak picking menu. The buttons function as follows:

2D Peak Picking Automatic Menu

Auto	Displays the 2D Peak Picking Automatic Menu (see below)
Edit	Displays the 2D Peak Picking Editing Menu (see below)
File	Displays the 2D Peak Picking File Menu (see below)
Display	Displays the 2D Peak Picking Display Menu (see below)
Return	Displays the 2D Display Main Menu (see page 117).

This menu provides automatic peak picking. The buttons functions as follows:

Box	The first button is labeled Box or Cursor, depending on the `dconi` display mode you are in. If labeled Box, you are in the `dconi` cursor mode, and this button changes the display to the `dconi` box mode with two pairs of cursors.
Cursor	If labeled Cursor, you are in the `dconi` box mode, and this button changes the display to the `dconi` cursor mode with one pair of cursors.
Peak	Automatically finds peaks in the 2D spectrum. If one cursor is visible (`dconi` cursor mode), all peaks above the current threshold in the currently displayed region of the spectrum are found and marked. A peak is defined as a data point that is higher than the eight points around it. Once such a point is found, the actual peak location is determined by interpolation in both dimensions.
Volume	Automatically finds the bounds of a peak and the integral of all points within these bounds. The bounds are found by descending down the sides of a peak until the point is reached where the amplitude of a data point is less than `th2d` times the current threshold. Thus, using a smaller value for `th2d` will cause `ll2d` to find and integrate a larger area for the bounds of the peaks. The peak volume is calculated by summation of all data points within these bounds. If the bounds of a peak already exist, the volume is recalculated.
Expand	The fourth button is labeled Expand or Full depending on the mode you are in. If labeled Expand, you are in the box mode and this button expands the area between the cursors.
Full	If labeled Full, you are in the cursor mode and this button displays the full area.
Both	Pick peaks and calculate volumes. The Both button does both the peak and volume operations at once.

Adjust	Adjust peak bounds so that none overlap. The Adjust button adjusts all peak bounds in the displayed region of the spectrum so that none overlap and recalculates peak volumes with the new peak bounds.
Reset	Deletes all peaks that have been found in the current spectrum.
Return	Display the 2D Peak Picking Main Menu (see above).

2D Peak Picking Edit Menu

This menu provides interactive peak editing. The buttons functions as follows:

Box	The first button is labeled Box or Cursor, depending on the display mode you are in. If labeled Box, you are in the cursor mode, and this button changes the display to he box mode with two pairs of cursors.
Cursor	If labeled Cursor, you are in the box mode, and this button changes the display to the cursor mode with one pair of cursors.
Mark	In `dconi` cursor mode, this button inserts a peak at the current cursor location. In `dconi` box mode, the cursors are taken as peak bounds and the area inside the cursors is integrated. These peak bounds are then assigned to all peaks within the cursors that do not already have their bounds defined. If a peak without bounds does not exist inside the area defined by the cursors, the highest point within that area is found, marked as a peak, and assigned the bounds defined by the cursors.
Unmark	In `dconi` cursor mode, this button deletes the peak nearest the cursor. In `dconi` box mode, this button deletes peak bounds from peaks whose bounds are entirely within the area defined by the cursors.
Expand	The fourth button is labeled Expand or Full depending on the mode you are in. If labeled Expand, you are in the box mode and this button expands the area between the cursors.
Full	If labeled Full, you are in the cursor mode and this button displays the full area.
Clear	In `dconi` cursor mode, this button deletes all peaks in the area of the spectrum displayed in `dconi`. In `dconi` box mode, the Clear button deletes all peaks that are within the area defined by the cursors.
Combine	This button works only in `dconi` box mode. It combines all peaks within the area defined by the cursors into a single peak. This combination peak is located at the average frequencies of all of the original peaks and has bounds that encompass all of the original bounds of the peaks. The volume of the combination peak is calculated by summation of all data points within its bounds. You may wish to back up the peak file using the Backup File button in the 2D Peak Picking File Menu (see below) prior to using this button, because the original peaks are permanently deleted when the combination peak is created.
Label	Prompts for a 15-character label to be assigned to the peak nearest the cursor (`dconi` cursor mode) or to all peaks within the area defined by the cursors (`dconi` box mode). Based on the value of the parameter `ll2dmode`, this label can be displayed next to the peak in `dconi`.

Comment	Prompts for an 80-character string to be assigned to the peak nearest the cursor (cursor mode) or to all peaks within the area defined by the cursors (box mode).
Info	Prints the peak file information about the peak nearest the cursor to the text window.
Set Int	Set the value of the peak volume.
Return	Display the 2D Peak Picking Main Menu (see above).

2D Peak Picking File Menu

Read	Prompts for the filename of a binary peak file and reads that file into VNMR. When a file is read in, the current peak file (`peaks.bin` for 2D spectra) is overwritten by a copy of the peak file that was read in.
Read Text	Prompts for the file name of a text peak file and reads that file into VNMR. When a file is read in, the current peak file (`peaks.bin` for 2D spectra) is overwritten by a new binary copy of the peak file that was read in.
Write Text	Prompts for a filename to write a text version of the current `ll2d` peak file.
Backup File	Prompts for a file name to copy the current binary peak file. It is a good idea to do this occasionally when doing a significant amount of interactive peak editing, so that intermediate versions of the peak file can be recovered in the event of an error (such as inadvertently selecting the Clear or Reset button or making a mistake using the Combine button).
Return	Display the 2D Peak Picking Main Menu (see above).

2D Peak Picking Display Menu

Sh Pk	The first button is labeled either Sh Pk (for “show peak”) or Hd Pk (for “hide peak”), which is used to select whether or not `dconi` automatically displays a “+” to mark each peak. If labeled Sh Pk, the “+” is hidden and this button shows a “+” at the location of each peak.
Hd Pk	If labeled Hd Pk, the “+” is shown and this button hides the “+” at the location of each peak.
Sh Num	The second button is labeled either Sh Num (for “show number”) or Hd Num (for “hide number”), which is used to select whether or not `dconi` automatically displays the peak number next to each peak. If labeled Sh Num, the peak numbers are now hidden and this button shows a peak number next to each peak.
Hd Num	If labeled Hd Num, the peak numbers are now shown and this button hides the peak numbers.
Sh Box	The third button is labeled either Sh Box (for “show box”) or Hd Box (for “hide box”), which is used to select whether or not `dconi` automatically displays the peak bounds of each peak. If labeled Sh Box, the box is now hidden and this button shows a box with the area integrated to get the volume of the peak.

Hd Box	If labeled Hd Box, the box is now shown and this button hides this box.
Sh Lbl	The fourth button is labeled either Sh Lbl (for “show label”) or Hd Lbl (for “hide label”), which is used to select whether or not `dconi` automatically displays the peak label next to each peak. If labeled Sh Lbl, the peak labels are now hidden and this button shows a peak label next to each peak.
Hd Lbl	If labeled Hd Lbl, the peak labels are now shown and this button hides peak labels.
Sh All	The fifth button is labeled either Sh All (for “show all”) or Hd All (for “hide all”), which is used to make `dconi` automatically display a “+”, the peak number, the peak bounds, and the peak label for each peak. If labeled Sh All, the “+”, number, box, and label are now hidden and this button shows “+”, number, box, and label for all peaks.
Hd All	If labeled Hd All, the “+”, number, box, and label are now shown and this button hides the “+”, number, box, and label for all peaks. The Hd All button is used to make `dconi` display no peak information.
Return	Display the 2D Peak Picking Main Menu (see above).

Automatic 2D Peak Picking

This section and the next few sections describe techniques for using the `ll2d` program.

Once a 2D spectrum has been Fourier transformed and the threshold has been adjusted to a suitable level in `dconi`, select the Peak button from the 2D Display Main Menu in `dconi` to display the 2D Peak Picking Main Menu. Then select the Auto button in that menu to display the 2D Peak Picking Automatic Menu.

If there are not many unwanted peaks visible above the threshold, then with one cursor showing (`dconi` cursor mode) selecting the Peak button will pick all peaks in the displayed region of the spectrum and write the frequencies and amplitude of each peak to the current peak file. If there are many artifacts or unwanted peaks in the spectrum, it is often better to either expand the display to show only regions of interest or select `dconi` box mode (two cursors showing) to enclose regions of interest with the cursors before selecting the Peak button. In `dconi` box mode, only peaks in the region enclosed by the cursors are picked.

As a general rule, it is more efficient to be selective when picking peaks and only pick peaks that you want, rather than picking all peaks and artifacts in a spectrum and later deleting the unwanted peaks.

Interactive Peak Picking or Editing

If unwanted peaks have been picked while using the 2D Peak Picking Automatic Menu, return to the 2D Peak Picking Main Menu and select the Edit button to display the 2D Peak Picking Edit Menu.

Individual peaks can be deleted by placing the cursor near the peak to be deleted and selecting the Unmark button. Groups of peaks can be deleted by entering the `dconi` box mode and enclosing the peaks to be deleted with the cursors prior to selecting the Clear button. The Clear button can also be used to delete all peaks in the currently displayed region of the spectrum if only a single cursor is visible (`dconi` cursor mode). A new peak can be interactively marked by placing the cursor in `dconi` cursor mode on a peak and

selecting the Mark button. In this case the peak location is set to be exactly at the cursor location. Subsequent automatic peak picking on the same peak will not necessarily coincide with peaks marked in this way.

Automatic Integration

Once peaks of interest have been picked, the peak bounds, full width at half-height (FWHH), and volume can be automatically determined using the Volume button in the 2D Peak Picking Automatic Menu. The volume is calculated by summing all data points within the bounds of the peak. The size of a peak's bounds that `ll2d` finds is determined by the current threshold and the value of the parameter `th2d`. If `th2d` is set to its maximum value of 1.0, the peak bounds will be selected so that they contain just the portion of the peak which is visible in `dconi` above the current threshold.

To make `ll2d` integrate a larger area, the value of `th2d` must be decreased. A value of `th2d` of 0.5, for example, will cause `ll2d` to find peak bounds that contain all portions of a peak greater than 0.5 times the current threshold in amplitude. Selecting too small of a value for `th2d` can cause extensive overlap of bounds of neighboring peaks. The Adjust button will attempt to adjust the bounds of all peaks within the displayed region of the spectrum, so that none overlap, and then recalculate peak volumes. In cases of extensive overlap of several peaks, however, this function may not always adjust the peak bounds in an optimal way.

Interactive Integration and Editing

The bounds of peaks can be interactively added or deleted using buttons in the 2D Peak Picking Edit Menu. To delete peak bounds, enclose the bounds of the peak(s) to be deleted entirely with the cursors in `dconi` box mode and select the Unmark button. This operation deletes the bounds, but not the peaks. To insert peak bounds on a peak without bounds, place the cursors in `dconi` box mode so they enclose the peak and the area to be integrated for that peak and select the Mark button. This mode can also be used to mark and integrate peaks. If the Mark button is selected when the two cursors in `dconi` box mode enclose a region where no peaks have yet been picked, it will find the highest point within the area defined by the cursors, interpolate, mark this as a peak, integrate the area inside the cursors, and assign these bounds and volume to the peak.

Peak information from the peak file can be written to the text window using the Info button in the 2D Peak Picking Edit Menu. This button writes out the peak table entry for the peak nearest the cursor in `dconi`. Adjust the value of the peak by using the Set Int button in the following manner:

1. Position the cursor over the peak.
2. Press the **Set Int** button.

   The program displays the prompt:

   ```
   Current integral is xx. New value?
   ```
3. Type in the value you want to assign to that peak.

Labeling and Commenting Peaks

The Label and Comment buttons in the 2D Peak Picking Edit Secondary Menu can be used to store additional information with a peak. The Label button prompts for a 15-character label to be assigned to the peak nearest the cursor (`dconi` cursor mode) or all peaks within the area defined by the cursors (`dconi` box mode). Assigning peak labels or comments to

a group of peaks in dconi box mode has no special significance in the ll2d program—the label or comment is simply stored in the peak file record of each peak in the group. The label can be displayed next to the peak in dconi based on the value of the parameter ll2dmode. An 80-character comment can be assigned to a peak or group of peaks using the Comment button. This comment is stored in the peak file with the peak entry and can contain any information desired.

Displaying Peaks in dconi

Peak information— a "+" to mark the peak top, the peak number, a box to mark the peak bounds, and the peak label—will be automatically displayed in dconi based on the value of the parameter 112dmode, as described above. The 2D Peak Picking Display Menu provides buttons to set ll2dmode for easy selection of which peak attributes are displayed in dconi or plotted with pll2d.

Peak File Manipulations

As described above, the default binary peak file for an experiment is stored in the ll2d subdirectory of the current experiment directory. The 2D Peak Picking File Menu provides buttons to read, write and backup peak files. The Backup button prompts for a file name to which the current default ll2d peak file should be copied. Unless a full UNIX (starting with a "/") is specified, the file is copied to the specified filename in the current working directory. If a full UNIX path is specified, the peak file is copied to the specified file in the specified directory. If no file name is specified at the prompt, the peak file is copied to the default peak filename with .bck appended (peaks.bin.bck for 2D peak files) in the current working directory.

The other buttons in this menu are used to read and write binary and text peak files. Each prompts for the name of a file, which is searched for by the following rules. If a full UNIX path is given, the specified file is read or written, otherwise a "read" searches for the file first in the current working directory and then in the ll2d subdirectory of the current experiment directory, while a "write" writes the file to the current working directory.

3.14 3D NMR

VNMR includes full support for 3D NMR, including acquisition, processing, and display. Table 29 and Table 30 list commands and parameters connected with 3D NMR.

Many of the 3D-related macros and parameters—for example, centersw2, cr2, crl2, delta2, dmg2, lp2, lsfid2, phfid2, rfl2, rfp2, rp2, sp2, wp2—are normally used in the same manner as their 1D and 2D counterparts and are not described further in this section.

In a non-arrayed 3D experiment, there are two implicitly arrayed parameters: d2 and d3. d2 is associated with ni and sw1, d3 with ni2 and sw2. The order of these two arrayed parameters is such that d2 is cycled the most rapidly.

In an arrayed 3D experiment, such as a single 3D with "superhypercomplex" data acquisition (States-Haberkorn method applied along both t_1 and t_2), there are, in reality, at least three arrayed elements. By convention, such an arrayed 3D experiment is implemented using four arrayed elements: d3 (t_1 evolution time), phase2, d2 (t_2 evolution time), and phase.

Table 29. 3D NMR Commands and Parameters (Part 1 of 2)

Commands	
addpar('3d')	Add selected 3D parameters to the current experiment
av2	Select AV mode on 2nd indirectly detected dimension.
centersw2	Move cursor to center of spectrum in 2nd indirect dimension
crl2	Clear reference line in 2nd indirectly detected dimension
dplane*	Display a 3D plane
dproj<(plane_type)>	Display a 3D plane projection
dsplanes*	Display a series of 3D planes
ft3d*	Perform a 3D FT on 3D FID data set (VNMR, UNIX)
getplane*	Extract planes from a 3D spectral data set
killft3d(exp_number)	Terminate any ft3d process started in an experiment
nextpl	Display the next 3D plane
par3d	Create 3D acquisition, processing, display parameters
ph2	Select phased mode on 2nd indirectly detected dimension
plplanes*	Plot a series of 3D planes
prevpl	Display the previous 3D plane
pwr2	Select power mode on 2nd indirectly detected dimension
resetf3	Reset parameters after a partial 3D Fourier transform
rl2<(frequency)>	Set reference line in 2nd indirectly detected dimension
set3dproc*	Set 3D processing
setsw2*	Setspectral width in 2nd indirectly detected dimension
wft*	Weight and Fourier transform 1D data
wft1d*	Weight and Fourier transform f_2 for 2D data
wft2d*	Weight and Fourier transform 2D data
wftt3	Process f3 dimension during 3D acquisition
wti<(element_number)>	Interactive weighting

```
* dplane(<plane_type,>plane_number)
  dsplanes(start_plane,stop_plane)
  ft3d<(<data_dir><,number_files><,'nocoef'><,plane_type>)> (VNMR)
  ft3d –e exp_number –f –r <options>  (UNIX)
  getplane<(data_directory><,place_directory><,plane_type>)>
  plplanes(start,stop<,'pos'|'neg'><,number_levels><,spacin  g>)
  set3dproc<(<'nocef'><,direectory>)>
  setsw2(nucleus,downfieldppm,upfieldppm):offset
  wft<(<options,><'nf'><,start><,finish><,step>)>,
  wft('inverse',exp_number,expansion_factor)
  wft1d(element_number), wft1d<(<options,><coefficients>)>
  wft2d<(<options,><coefficeints)>
```

(Continued on next page)

Assuming that `array='phase,phase2'` (see below), the order of arrays is such that the `phase2` array is cycled the most rapidly, followed by the `phase`, `d2`, and `d3` arrays.

3D Acquisition

3D data acquisition is accomplished with pulse sequences using the parameter `d3`, which is incremented according to the parameters `ni2` and `sw2`. This is analogous to `d2`, which is incremented according to `ni` and `sw1` for 2D NMR (of course, `d2`, `ni`, and `sw1` are active in 3D as well). In addition, the parameter `phase2` is used to control the "mode" of acquisition (hypercomplex, TPPI, or absolute value) in the third frequency domain, just like `phase` in the second domain. All of these 3D parameters are created with the macro

Table 30. 3D NMR Commands and Parameters (Part 2 of 2)

Parameter	
`cr2` {number}	Cursor position along 2nd indirectly detected dimension
`d3` {number, in sec}	Incremented delay for 2nd indirectly detected dimension
`delta2` {pos. number,in Hz}	Cursor difference in 2nd indirectly detected dimension
`dmg2` {'ph2','av2','pwr2'}	Display mode along 2nd indirectly detected dimension
`f1coef`	Coefficient to construct F1 interferogram
`f2coef`	Coefficient to construct F2 interferogram
`fiddc3d` {3-char string}	3D time-domain dc correction
`index2` {0, 1 to fn/2}	Projection or 3D plane index selected
`lp2` {–3600 to +3600,in deg.}	First-order phase in 2nd indirectly detected dimension
`lsfid2` {'n',number}	Number of complex points to left-shift ni2 interferogram
`lsfrq2` {number,in Hz}	Frequency shift of the fn2 spectrum, in Hz
`ni` {number}	Increments in 1st indirectly detected dimension
`ni2` {number}	Increments in 2nd indirectly detected dimension
`path3d` {path}	Path to currently displayed 2D planes from a 3D data set
`phfid2` {number}	Phase selection for 3D acquisition
`phfid2`*	Zero-order phasing constant for ni2 interferogram
`plane`*	Currently displayed 3D plane type
`ptspec3d` {3-char string}	Region-selective 3D processing
`rfl2` {number,in Hz}	Reference peak position in 2nd indirectly detected dimension
`rfp2` {number,in Hz}	Reference peak frequency in 2nd indirectly detected dimension
`rfp2` {–360.0 to +360.0,in deg.}	Zero-order phase in 2nd indirectly detected dimension
`sp2` {number,in Hz}	Start of plot in 2nd indirectly detected dimension
`ptspec3d` {3-char string}	Flag for 3D spectral dc correction
`scalesw2` {'n',n > 0}	Scale spectral width in 2nd indirectly detected dimension
`sw2` {number, in Hz}	Spectral width in 2nd indirectly detected dimension
`trace` {'f1','f2','f3'}	Mode for *n*-dimensional data display
`wp2` {number, in Hz}	Width of plot in 2nd indirectly detected dimension
* `plane` {'f1f3','f3f1','f2f3','f3f2','f1f2','f2f1'}	
`phfid2` {'n',–360.0 to +360.0,in deg}	

`addpar('3d')` along with other 3D parameters, including `fiddc3d` for 3D time-domain dc correction, `ptspec3d` for region-selective 3D processing, and `path3d` for the path to the currently displayed 2D planes extracted from a 3D data set. (The macro `par3d` is functionally equivalent to `addpar('3d')`.)

By convention, 3D sequences are described with the first evolution time being known as t_1, the second evolution time as t_2, and the time during which data are acquired as t_3. After transformation, these same dimensions are called the f_1, f_2, and f_3 dimensions.

3D Processing

Data processing includes the `ft3d` command for full 3D processing, governed by the usual parameters to control transform sizes, weighting, phasing, etc., with a “2” at the end of the parameter name signifying the third dimension. Unlike other VNMR commands, `ft3d` occurs in the background by default; that is, it is run as a separate task by UNIX, leaving VNMR free to continue with other tasks (including 1D and 2D processing of the same data set!). To increase the speed of 3D transforms further, the `wftt3` macro allows the software to process one dimension (the acquisition or t_3 dimension) as the data are being acquired. Also, the `ft3d` software can be configured to run on several computers simultaneously, for

even greater speeds. The killft3d macro terminates any ft3d program that has been started in an experiment.

3D Display

Once the data are processed, the data can be displayed as two-dimensional planes of the 3D data set in any of the three orthogonal directions. Skew planes are not supported, nor are "full 3-dimensional" displays. One command, getplane, extracts the 2D planes from the 3D data set in one or more of the three orientations. After the planes are "extracted" in this manner, they are displayed with the dplane macro. The parameter index2 keeps track of which plane is on display. The macro nextpl displays the next plane from the plane currently on view. Another macro, prevpl, shows the previous plane from the current plane.

The dsplanes(start_plane,stop_plane) macro produces a graphical 2D color or contour map for a subset of 3D planes specified by the arguments. The dconi program is used to display the planes. The plplanes macro is available to plot a series of 3D planes.

The new concept of time-domain frequency shifting can be employed to good use in 3D NMR, where spectra in the indirectly detected directions are often "folded" by accident or by choice. The parameters lsfrq, lsfrq1, and lsfrq2 cause the frequency of the spectrum to be shifted as part of the Fourier transformation process.

3D Pulse Sequences

No standard and fully documented pulse sequences are provided for 3D NMR in the released software, although a number of sequences will be found in the user library. If you are writing your own sequences, you simply need to write a sequence that includes a d2 and d3 delay (these delays may also be d2/2 or d3/2). If your sequence is to operate in the hypercomplex (or the hyper-hypercomplex) mode, you should use the parameters phase and phase2 to select between the two orthogonal components of the hypercomplex experiment in the relevant domain. To ensure that your experiment is processed correctly using the default processing coefficients, you should write your pulse sequence so that the phase=2 (and phase2=2) experiments leave the receiver unchanged (compared to phase=1) and either increment the phase of the pulse (or pulse sandwich) just prior to the relevant evolution, or decrement the phase of the pulse following evolution by 90 degrees (or for multiple-quantum experiments, by 90/*n*).

Experiment Setup

Setup is necessary in 3D experiments to position transmitters and decoupler, adjust pulse widths, etc. Just as the setup of 2D experiments can often be assisted by performing "first increment" experiments (i.e., a 1D experiment that represents the first increment of the 2D), so 3D experiments can be assisted not only by 1D setup experiments, but also by "first plane" 2D experiments (not available on *GEMINI 2000* systems). To perform a 2D experiment in the sw1 dimension, set ni2=1 and phase2=1, with ni greater than 1 and phase=1,2 (or phase=3 for TPPI experiments). This combination of parameters will perform a "normal" 2D experiment, incrementing d2, and the data can be processed with the wft2da command (or its variants).

The "third dimension" 2D experiment is performed by setting ni=1 and phase=1, with ni2 greater than 1 and phase2=1,2 (or phase2=3, as desired). These parameters will produce a 2D experiment in which d3 is incremented, resulting in a spectral width sw2.

The wft2d command must be given the special argument ni2 to process this data correctly, for example, wft2d('ni2',1,0,0,0,0,0,-1,0). You cannot use the wft2da('ni2') because the wft2da macro does not support this argument.

Notice that when you process a "first plane" 2D experiment, the axes are always labeled f_1 and f_2 because this is considered to be a 2D experiment, and hence the axis labeling corresponds to conventions used in 2D NMR.

When you are finished setting up the 3D experiment, reset ni, ni2, phase, and phase2 to their desired values. Check the value of the parameter array and make sure that array='phase,phase2' and not 'phase2,phase', which will acquire data in the incorrect order. To ensure the correct order, always enter phase before phase2, or simply enter array='phase,phase2'.

Data Processing

Just like processing 2D NMR, the proper processing of 3D NMR requires coefficients to select various components of the data to be combined to form the final data set. There are actually up to 40 coefficients required that are explained in more detail in the *VNMR Command and Parameter Reference*. In normal operation, the coefficients will be transparent to you, just as the 2D coefficients are. The set3dproc command can create a 3D coefficient file for processing 3D FID data under certain conditions.

The ft3d command determines from the values of phase and phase2 what the expected coefficients are, based on whether a hypercomplex ("States-Haberkorn") or TPPI experiment has been performed in a particular dimension. This assumes that the pulse sequence has been written to perform "standard" phase cycling as described above. If your data are reflected along a particular dimension, it is possible (or probable) that different coefficients are required for data processing. In this case, the ft3d('nocoef') form is used to allow you to specify you own coefficients (which are found in a text file named coef in the 3D experiment directory, unlike in ft2d, where they are given as arguments to the command). By default, ft3d calls the make3dcoef macro to create a coefficient file using the f1coef and f2coef string parameter values.

The format for the 3D coefficient file is an extension of that used for 2D coefficients. The coefficient file contains four rows of eight coefficients used to construct the t_2 hypercomplex interferograms and a final row of eight coefficients used to construct the t_1 interferogram. The actual values of the coefficients depends on the order in which the States-Haberkorn components of the 3D FID data set were collected. This order depends in turn on the values of the parameters phase, phase2, and array.

If TPPI phase cycling is used to collect data along one or both of the indirectly detected dimensions, instead of four data sets per (ni,ni2) increment, there are only two or one data sets, respectively, per (ni,ni2) increment. If there are only two data sets per (ni,ni2) increment, the coef file contains four rows of four coefficients that are used to construct the t_2 hypercomplex interferograms, and a final row of eight coefficients that are used to construct the t_1 interferogram. If there is one data set per (ni,ni2) increment, the coef file contains four rows of two coefficients that are used to construct the t_2 hypercomplex interferograms and a final row of eight coefficients that are used to construct the t_1 interferograms.

Phasing a 3D data set is best accomplished using 2D transforms. In general, the recommended method in writing 3D pulse sequences is to attempt to minimize frequency-dependent phase shifts in f_1 and f_2. Even so, there are generally small phase shifts that must be dealt with. The following steps are suggested:

1. Set **pmode='full'** to allow full phasing in both dimensions after a 2D transform.

2. Adjust **rp** and **lp** on a 1D spectrum (the first increment of the 3D), just as you would for 2D (e.g., by typing `wft(1)`).
3. Enter **`wft2d('ni',1,1,0,0,0,0,0,0,0,0,0,0,–1,0,0,0)`** to adjust f_1 phasing (there are 11 consecutive zeros in the middle of this argument).
4. You now have an f_1f_3 2D spectrum (with incorrectly labeled axes). Set **`trace='f1'`** to adjust the f_1 phase, then set **`trace='f2'`** to trim the f_3 phasing. You can now adjust **`rp1`** and **`lp1`** (as well as `rp` and `lp`).
5. Enter **`wft2d('ni2',1,1,0,0,0,0,0,0,0,0,–1,0,0,0,0,0)`** to adjust f_2 phasing (note that this argument has nine consecutive zeros in the middle and five zeros at the end).
6. You now have an f_2f_3 2D spectrum. Set **`trace='f1'`** to adjust the f_2 phasing (**`rp2`** and **`lp2`**), then set **`trace='f2'`** to trim the f_3 phasing if necessary.

One additional point on phasing. Some pulse sequences are written to result in a 180° phase shift across the spectrum. Remember that in VNMR, the "origin" for phasing is defined as the right edge of the spectrum; however, in "real" terms, the actual origin of phasing (i.e., the zero-frequency point) is at the center of the spectrum. Thus, if you expect a certain `lp1` or `lp2` value, such as –180°, you should simultaneously use a value of `rp1` or `rp2` equal to `–lp1/2` or `–lp2/2` (e.g., 90°).

If you want to adjust the weighting functions for the 3D transform by using the `wti` command and examine interferograms, you can do so along either the t_1 or t_2 axes. Use the same commands given above to adjust the phasing (the commands with the long series of zeros), but use `wft1d` instead of `wft2d`.

For the final transformation, the `specdc3d` parameter controls the dimensions in which a spectral drift correction is performed on the data. A three-letter value of `'ynn'` gives drift correction along f_3 (the first letter) but not along f_1 (the second letter) or f_2 (the third letter); this value is probably a good starting point for your efforts.

The `pmode` parameter is ignored by the 3D transformation; no phasing is possible after the 3D transform.

The 3D transformation process needs to be followed by the process of extracting the 2D planes from the full 3D data set. This can be done separately, with the `getplane` command, but most often is combined with the `ft3d` command. In general, and especially for heteronuclear experiments, the f_1f_3 and f_2f_3 planes are the most interesting. The f_1f_2 plane is not only generally less useful, but also is considerably slower to extract from the data. The recommended command to use for 3D transformation, therefore, is `ft3d('f1f3','f2f3')`, which performs the 3D transform and extracts the two interesting planes in one step.

Solvent suppression works on t_3 FIDs of 3D spectra just like in the 1D and 2D cases.

Following the transform, set `plane='f1f3'` or `'f2f3'` and then use the `dproj` macro to display the projection of the data on that plane, or `dplane(n)` to display the nth plane. The `resetf3` macro will reset parameters after a partial 3D Fourier transform.

3.15 4D NMR Acquisition

The `addpar('4d')` macro creates the parameters `ni3`, `sw3`, `d4`, and `phase3` that can be used to acquire a 4D data set (the macro `par4d` functions the same as `addpar('4d')`).

The parameter `ni3` is the number of t_2 increments, `sw3` is the spectral width along the third indirectly detected dimension, `d4` is the incremented delay, and `phase3` is the phase selection for 4D acquisition. Processing and display in 4D is currently not available in VNMR.

Table 31 summarizes 4D acquisition commands and parameters.

Table 31. 4D NMR Acquisition Commands and Parameters

Commands	
addpar('4d')	Add 4D parameters to the current experiment
`par4d`	Create 4D acquisition parameters
Parameters	
`d4` {number,in sec}	Incremented delay for 3rd indirectly detected dimension
`ni3` {number}	Number of t_2 increments in 4D acquisition
`phase3` {number}	Phase selection for 4D acquisition
`sw3` {number,in Hz}	Spectral width along the 3rd indirectly detected dimension

Chapter 4. Multidimensional and Advanced Experiments

Sections in this chapter:

- 4.1 “Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY,” page 138
- 4.2 “COSY—Correlated Spectroscopy,” page 141
- 4.3 “COSYPS—Phase-Sensitive COSY,” page 141
- 4.4 “DQCOSY—Double-Quantum Filtered COSY,” page 143
- 4.5 “HET2DJ—Heteronuclear 2D-J,” page 145
- 4.6 “HETCOR—Heteronuclear Chemical Shift Correlation,” page 147
- 4.7 “HETCORPS—Absolute-Value and Phase-Sensitive HETCOR,” page 149
- 4.8 “HOM2DJ—Homonuclear J-resolved 2D,” page 150
- 4.9 “INADEQUATE—Double-Quantum Transfer Experiment,” page 152
- 4.10 “MQCOSY—Multiple-Quantum Filtered COSY,” page 153
- 4.11 “NOESY—Nuclear Overhauser Effect Spectroscopy,” page 155
- 4.12 “ROESY—Rotating Frame Overhauser Effect Spectroscopy,” page 158
- 4.13 “TNCOSYPS—COSYPS with Water Suppression,” page 160
- 4.14 “TNDQCOSY—DQCOSY with Water Suppression,” page 160
- 4.15 “TNMQCOSY—MQCOSY with Water Suppression,” page 161
- 4.16 “TNNOESY—NOESY with Water Suppression,” page 161
- 4.17 “TNROESY—ROESY with Water Suppression,” page 162
- 4.18 “TNTOCSY—TOCSY with Water Suppression,” page 163
- 4.19 “TOCSY—Total Correlation Spectroscopy,” page 164
- 4.20 “TROESY—Transverse ROESY,” page 166:
- 4.21 “HCCHTOCSY Pulse Sequence,” page 166
- 4.22 “HMQCTOCSY Pulse Sequence,” page 168
- 4.23 “HMQC-TOCSY 3D Pulse Sequence,” page 168
- 4.24 “HSQC-TOCSY 3D Pulse Sequence,” page 169

In these experiments, each pulse sequence has a macro, usually with the same name as the pulse sequence, that sets up the parameters for the experiment and then displays information on the experiment. The macro retrieves parameters such as `pw90`, `tpwr`, `dmf`, etc. from a central location like `/vnmr/probe` or `$vnmruser/probe`. Other specific parameters, such as `mix` for NOESY, come from `/vnmr/parlib`.

It is important that these parameters be correct. The first time the macro for an experiment is entered (e.g., by typing `noesy`), the system retrieves the default parameters and values.

To change any of these values (e.g., the default `mix` time in `noesy` is 0.2 seconds and you want the default to be 0.5 seconds), make the appropriate change in the displayed parameters, and then save the modified parameters either in your user's `parlib` or in the system `/vnmr/parlib`. Notice that because files in the directory `/vnmr/parlib` are available to all users, only the system administrator `vnmr1` has permission to save the files in this directory.

To view complete listings of each pulse sequence, print out or look at the contents of the files in the directory `/vnmr/psglib` on your system disk. You can also enter e `dps` to view a graphical representation.

The 2D pulse sequences HMQC (Heteronuclear Multiple-Quantum Coherence) and HMQCR (HMQC in Reverse Configuration) are described in Chapter 5, "Indirect Detection Experiments," of this manual.

4.1 Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY

The `relayh` macro sets up parameters for absolute-value COSY, single RELAY-COSY, or double RELAY-COSY pulse sequences. Figure 26 is a diagram of the absolute-value COSY sequence, and Figure 27 is a diagram of RELAY-COSY, single and double.

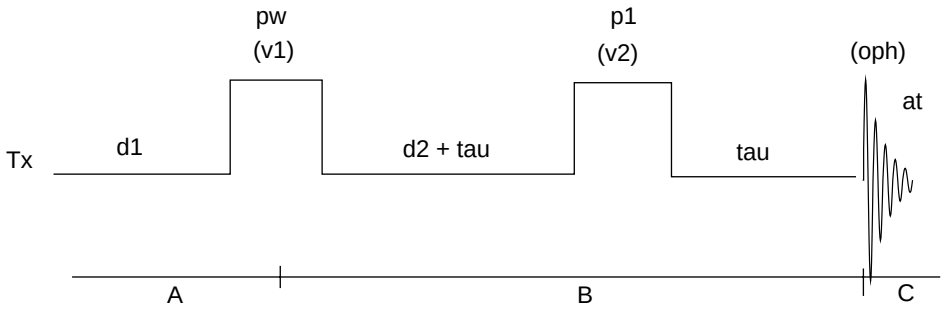


Figure 26. Absolute-Value COSY Pulse Sequence

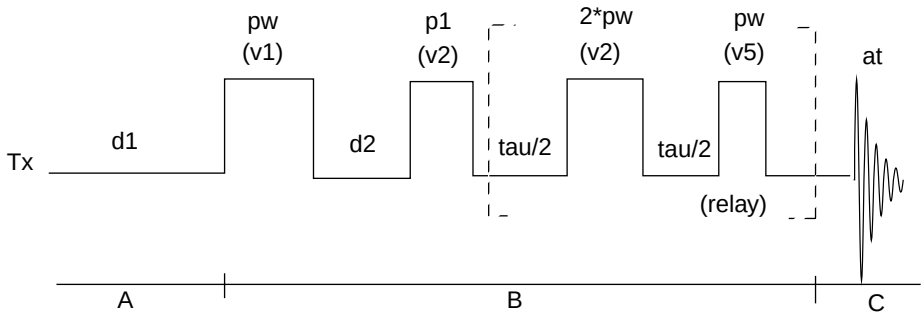


Figure 27. RELAY- COSY Pulse Sequences

Applicability

These sequences are available on all systems.

Parameters

`relay` is the number of relays to be performed:
if `relay=0`, a normal absolute value COSY is performed.
if `relay=1`, a RELAY-COSY is performed.
if `relay=2`, a double-RELAY-COSY is performed.

`pw` is a 90° pulse.

`p1` is a 90° pulse if `relay` is not equal to 0; if `relay` is equal to 0, diagonal peaks can be deemphasized by using pulses greater than 90° when doing P-type peak selection or by using pulses less than 90° when doing N-type peak selection.

`d1` is a delay set to 1 to 3 times T_1.

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (`sw1=sw`).

`v1`, `v2`, and `v5` are pulse phases. `oph` denotes the phase of the receiver.

`ni` is the number of t_1 increments (set so that `sw1/ni` equals 6 by default). If speed is essential, `ni` can be decreased.

`tau` is the propagation time for long-range COSY (`relay=0`) and for relayed COSY (`relay` greater than 0).

Technique

The COSY experiment, as implemented on UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, UNITY*plus*, *GEMINI 2000*, and UNITY systems, can be run several different ways. The experiment macro called is `relayh`. If the parameter `relay` is set to 0 (the default), a normal COSY is performed.

The standard setup uses two 90° pulses and is the traditional way to run COSY experiments. The second pulse can be changed to a 45° pulse to decrease the size of the diagonal. This technique is called “COSY-45.” Besides decreasing the diagonal, COSY-45 emphasizes active coupling partners relative to passive couplings so if sufficient digital resolution is present, the crosspeaks may show an interesting multiplet structure. To emphasize long-range couplings, increase the setting of the parameter `tau` to 0.2 seconds.

To set up and acquire:

1. Set up by acquiring a 1D proton experiment in `expn`, where `n` is 1, 2, 3, etc.
2. Narrow the ^{1}H spectral window to leave approximately 1 ppm on either side of the peaks of interest by using two cursors and entering **`movesw`**.
3. Reacquire the ^{1}H with the new spectral window.
4. Phase the ^{1}H spectra.
5. Move the FID to another experiment (e.g., to move the FID from `exp1` to `exp2`, enter `mf(1,2))`.

6. Enter **relayh** to modify the parameters for the RELAYH experiment. The relayh macro calculates appropriate weighting functions.
7. Turn the spinner off.
8. Enter **au** to acquire data.

To Fourier transform:

1. Enter **wft2d**. This performs weighted Fourier transforms in both dimensions and displays the data as a contour map.
2. Adjust the threshold and vertical scale.

To interactively adjust weighting during processing:

1. Enter **wft(1)** to Fourier transform the first increment of data.
2. Enter **wti** to start interactive weighting.
3. Absolute-value data is usually processed using sinebell weighting. This is the default weighting function calculated by the macro setting up the experiment. Adjust the sinebell so that data decays to zero before the end of the window. Adjusting the weighting function on the first increment of data sets the weighting function in the 2D time dimension t_2.
4. Enter **wft1d** to Fourier transform the t_2 dimension. A contour map of f_2,t_1 is displayed, showing the individual interferograms.
5. Click on the **trace** button and choose a trace through one of the horizontal interferograms.
6. Enter **wti** to bring up interactive weighting of the interferogram. Adjust the weighting function the same as before.
7. Enter **wft2d** to complete the Fourier transformation.
8. Enter **foldt** to symmetrize the data.
9. You can adjust the vertical scale and threshold to provide a better display in which the cross peaks and diagonal are more easily seen. Adjust the vertical scale by changing the value of the parameter **vs** (e.g., vs=12).

   If the vertical scale is very high, you can decrease it by typing vs=vs/10 (or a similar divisor) to drop the vertical scale by that amount. Adjust the threshold by using the mouse to adjust the sliding color scale beside the 2D contour plot.
10. Enter **dcon** to display a non-interactive color intensity map, or enter dconi to permit interaction.
11. Enter **dpcon(12,1.3)** to see what your data will look like if plotted with 12 contours spaced 1.3 levels apart. If you wish, try other arguments for dpcon to see how other values of contours and levels will look when plotted.
12. Enter **plcosy(8,1.5)** to plot the COSY or RELAYH data with eight contours spaced 1.5 levels apart. Try other values if you want. The macro plcosy actually takes three arguments: the number of contours, the spacing of levels, and the experiment number that contains the 1D data. If there is no third argument, plcosy assumes that the 1D data is in experiment 1. Therefore, plcosy(8,1.5) plots the contour, retrieves the 1D data, and plots it above the contour.
13. Enter **dconi** to make an expansion of the data and redisplay the contour map if it is not present. Expand around the upfield region by using the left mouse button to

determine the lower left corner and the right mouse button to determine the upper right corner, then click the expand button.

14. Click on the **expand** button.
15. Enter **plcosy** to plot this expanded region. Note that the appropriate 1D region is plotted on the top and to the side of the 2D plot.

Potential Problems

The COSY experiment is very forgiving and usually works (to a greater or lesser extent) with a slightly incorrect 90° pulse width. The most common reason for failure of the experiment is that the value for `p1` is incorrect. Make sure that the 90° pulse is correct before beginning the experiment. Remember that `pw`, the second pulse, may be set to 45° to deemphasize the diagonal and provide a filter for passive couplings.

A second problem that can arise are artifacts caused by pulsing too rapidly. If you symmetrize the spectrum with `foldt` (normal in COSY), these should be minimized.

4.2 COSY—Correlated Spectroscopy

The `cosy` macro converts a parameter set to a COSY experiment.

Applicability

COSY is available on all systems.

Parameters

For information on COSY parameters, refer to page 138.

Technique

To set up, acquire, and process, use the technique given in "Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY," page 138.

4.3 COSYPS—Phase-Sensitive COSY

The `cosyps` macro sets up a phase-sensitive COSY pulse sequence. Figure 28 is a diagram of the COSYPS sequence.

Applicability

COSYPS is available on all systems.

Parameters

`pw` is a 90° pulse.

`p1` is 90° if `phase` is not equal to `0`; if `phase` equals `0`, pulses greater than 90° de-emphasize diagonal peaks for P-type peak selection.

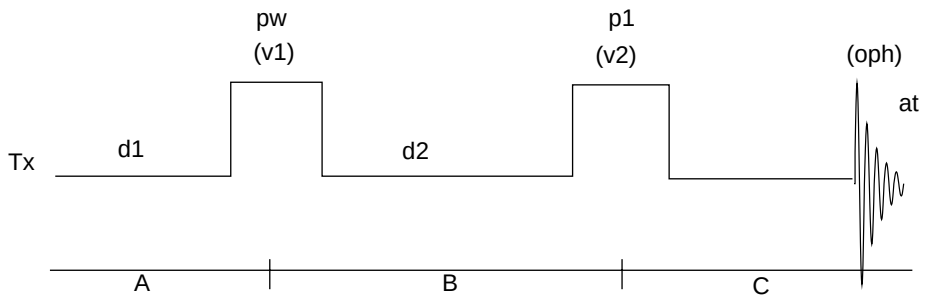


Figure 28. COSYPS Pulse Sequence

`d1` is set to equal 1 to 3 times the value of T_1.

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is spectral width (usually `sw1=sw`, except for `phase=3`).

`ni` is the number of t_1 increments (by default `sw1/ni=12`); it may be decreased if speed is essential.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm` but does depend on `dmm` and is activated as a part of `d1` if `presat` is greater than 0. `presat` is available on *MERCURY-VX* and *MERCURY* systems with *GLIDE* (uppercase pulse sequences only). `presat` is not active on *GEMINI 2000* systems.

`phase=1,2` (suggested value) for phase-sensitive data. `phase=0` for 2D data in an *av* display (P-type peaks). `phase=1,2` for 2D hypercomplex data (States-Haberkorn method). `phase=3` for 2D TPPI data. For `phase=3`, remember that `sw1=2*sw`.

`nt` is multiple of 8 (minimum, `phase=0`) or multiple of 4 (minimum, `phase=1,2` or `3`).

Notice that for all TPPI experiments, the resulting spectrum appears doubled. Just display and phase one-half of the data.

Phase Cycling

`v1` is the phase for first pulse.
`v2` is the phase for second pulse.
`oph` is the phase for the receiver.

```
v1  = x –x y –y y –y x –x
v2  = x  x y  y y  y x  x
oph = x –x y –y y –y x –x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add 90∗(`ix` – 1)° to `v1`, where `ix` is the increment counter. For `phase=0`, the subcycle of P-type peak selection is mixed into `v2`.

Technique

To set up and acquire data, use the technique given in "Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY," page 138.

To process the data, enter `wft(2)` to display the first increment with `phase=2` in t_1, phase it to be pure absorptive, then add 90° to `rp`. Set `rp1=90` and `lp1=0` initially. If the f_1 phasing is not satisfactory, adjust `rp1` and `lp1` so that the diagonal peaks are pure absorptive and then add 90° to `rp1`.

References

Bodenhausen, G.; Freeman, R.; Niedermeyer, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *26*, 133–164.

Bachmann, P.; Aue, W. P.; Muller, L; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 29–39.

4.4 DQCOSY—Double-Quantum Filtered COSY

The `dqcosy` macro sets up parameters for the DQCOSY pulse sequence. Figure 29 is a diagram of the sequence.

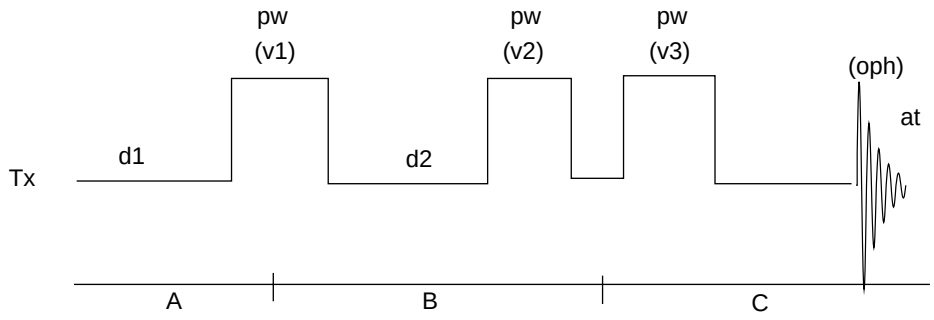


Figure 29. DQCOSY Pulse Sequence

Applicability

DQCOSY is available on all systems.

Parameters

`pw` is a 90° pulse on the observed nucleus.

`d1` is a relaxation delay (1 to 3 times the value of t_1).

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (usually `sw1=sw`, except for `phase=3`).

`ni` is the number of t_1 increments (set so that `sw1/ni=12`).

`phase=0` for 2D data in an `av` display (P-type peaks); `phase=1,2` for 2D hypercomplex data (States-Haberkorn method); `phase=3` for 2D TPPI data.

`sspul='y'` activates a homospoil–90–homospoil sequence that precedes `d1`; this is used to achieve a less oscillatory steady-state for 2D experiments where the recycle time is shorter than t_1. `sspul` is not active on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm`, but does depend on `dmm`, and is activated as a part of `d1` if `presat` is greater than 0. `presat` is available on *MERCURY-VX* and *MERCURY* systems with *GLIDE* (uppercase pulse sequences only). `presat` is not active on *GEMINI 2000* systems.

`nt` is a multiple of 8 (`phase=0`) (minimum) or multiple of 4 (`phase=1,2`, or `3`) (minimum).

Phase Cycling

`v1` is the phase for first `pw` pulse.
`v2` is the phase for second `pw` pulse.
`v3` is the phase for third `pw` pulse.
`oph` is the phase for receiver.

```
v1 =  x  x  x  x  y  y  y  y  x  x  x  x  y  y  y  y
     -x -x -x -x -y -y -y -y -x -x -x -x -y -y -y -y
v2 =  x  x  x  x  y  y  y  y -x -x -x -x -y -y -y -y
v3 =  x  y -x -y  y -x -y  x
oph=  x -y -x  y  y  x -y -x -x  y  x -y -y -x  y  x
     -x  y  x -y -y -x  y  x  x -y -x  y  y  x -y -x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add 90∗ (`ix` – 1)° to `v1`, where `ix` is the increment counter. For `phase=0`, the subcycle of p-type peak selection is added in after the basic four-step cycle of `v3`.

Technique

To set up and acquire data, use the technique given in “Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY,” page 138.

No phasing should be necessary for processing. For full processing, use menus or enter `wft2da`. For processing, proceed as follows:

1. Enter **`wft(1)`** to Fourier transform the first increment of data. There should be no signals in the first increment of a DQCOSY.
2. Enter **`wti`** to start interactive weighting. Data from DQCOSY is phase-sensitive and usually processed using Gaussian weighting, which is the default weighting function. Adjust the Gaussian weighting so that the data decays to zero before the end of the window. Adjusting the weighting function on the first increment of the data sets the weighting function in the 2D time dimension t_2.
3. Enter **`wft1da`** to Fourier transform the t_2 dimension and display a contour map of f_2,t_1 showing individual interferograms. Click on the trace button and choose a trace through one of the interferograms. Enter **`wti`** to bring up interactive weighting of the interferogram. Adjust the weighting function as before.

4. Enter **wft2da** to complete the Fourier transformation.
5. Display and plot using the procedures described in "Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY," page 138.

Potential Problems

The DQCOSY experiment is much more sensitive to artifacts than the COSY experiment. DQCOSY experiments are not symmetrized so t_1 noise is a much greater problem. To minimize t_1 noise, make sure that the 90° pulse is correct before beginning the experiment. Another problem that can arise is the presence of artifacts due to pulsing too rapidly. Make sure you set `d1` to at least 1 to 3 times the T_1 value of the protons in the sample. Run the sample nonspinning and use homospoil pulses (homospoil is not available on *GEMINI 2000* systems).

References

Piatini U.; Sorenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.

Rance, M. et al *Biochem. Biophys. Res. Comm*. **1983**, *117*, 479–485.

4.5 HET2DJ—Heteronuclear 2D-J

The `het2dj` macro sets up parameters for the HET2DJ experiment. Absolute-value (`av`) mode is required. The experiment can be performed either in a gated or a non-gated mode. Figure 30 is a diagram of the sequence.

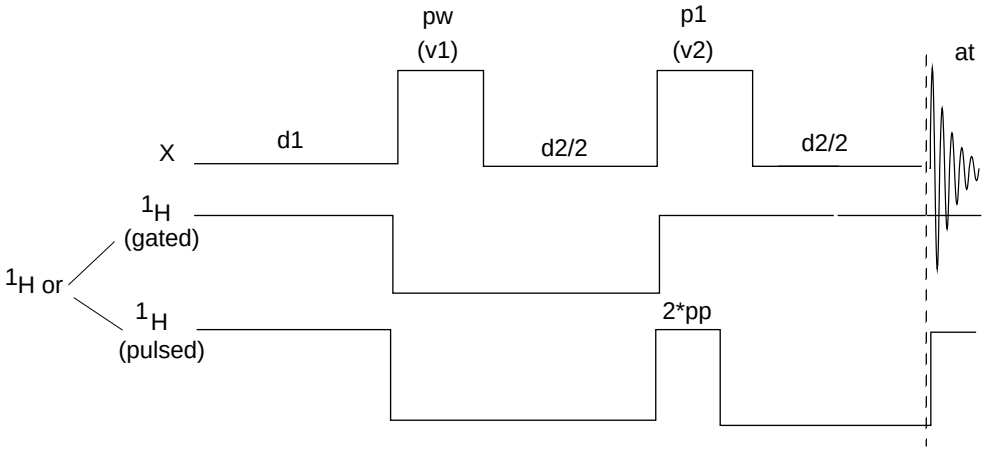


Figure 30. HET2DJ Pulse Sequence

Applicability

HET2DJ is available on all systems.

Parameters

`pw` is the observe 90° pulse (X nucleus).

`pp` is the proton 90° pulse width supplied from decoupler.

`p1` is the observe 180° pulse (if `p1=0`, `2*pw` is used).

`tpwr` is the power level for observe pulses (systems with linear amplifiers).

`pplvl` is the power level for proton pulses (systems with linear amplifiers).

`sw1` should cover the maximum multiplet pattern.

`dm='ynyy'` for decoupler gating during the evolution period. `dm='ynny'` for no decoupler gating during the evolution period (proton flip experiment).

`dmm='wcw'` (WALTZ) or `dmm='fcf'` (no WALTZ).

`nt` is a multiple of 2 (minimum) or a multiple of 16 (maximum and recommended). Note that 1 is possible.

If decoupler gating is used during the evolution period, the actual J value is twice the measured J (`Jact = 2*Jmeas`).

Technique

1. A good sample to try the first time is 30% menthol in $CDCl_3$. This sample is concentrated enough that good data can be obtained in a short period of time.
2. Enter **`jexp2`** to join experiment 2 (this can also be done from the Workspace menu).
3. Set up a carbon experiment by clicking the **C13,CDCl3** button in the setup menu.
4. Acquire a ^{13}C spectrum.
5. Enter **`mp(2,3) jexp3`** to move the parameters from experiment 2 to experiment 3 and join experiment 3.
6. Enter **`het2dj`** to set up the HET2DJ experiment.
7. Enter **`go`** to acquire the data.
8. Enter **`wft2d`** to Fourier transform.
9. After transformation, the data is tilted along a 45° angle. To rotate the data, enter **`rotate(45.0)`**.
10. Enter **`foldj`** to symmetrize the data.
11. Display the data the same as described in “COSY—Correlated Spectroscopy,” page 141.
12. Enter **`pcon(8,1.5) page`** to plot the data with 8 contours spaced 1.5 levels apart. If you wish, try other values for the arguments to see various plots.

    The `pcon` command actually takes three arguments: positive or negative, the number of contours, and the spacing of levels. Positive or negative refers to the ability to plot exclusively positive or negative contours. The default is to plot both positive and negative contours. Since the HET2DJ data has no negative contours, this option can be ignored.

Potential Problems

The most common reason for failure of the HET2DJ experiment is that the values for `pp` and `pw` are incorrect. Make sure that the 90° pulse on the decoupler, `pp` and the 90° pulse on ^{13}C, `pw` are correct before beginning the experiment.

Another possible problem is the presence of artifacts due to pulsing too rapidly. This experiment is carbon detected. Make sure you set `d1` to a value that permits sufficient relaxation, the T_1 value of the protonated carbons.

References

Bodenhausen, G.; Freeman, R.; Turner, D. *J. Magn. Reson.* **1977**, ***27***, 511.

Freeman, R.; Keeler, J. *J. Magn. Reson.* **1981**, ***43***, 484–487.

4.6 HETCOR—Heteronuclear Chemical Shift Correlation

The `hetcor<(exp_number)>` macro sets up parameters for HETCOR pulse sequence, where the optional argument `exp_number` is the number of the experiment, from 1 through 9, in which a proton spectrum of the sample already exists, (e.g., `hetcor(2)` specifies experiment 2).

`hetcor` includes a presaturation option, composite 180° pulses, and simultaneous pulses on transmitter and decoupler rf channels. Figure 31 is a diagram of HETCOR set for decoupled multiplets (`hmult='n'`).

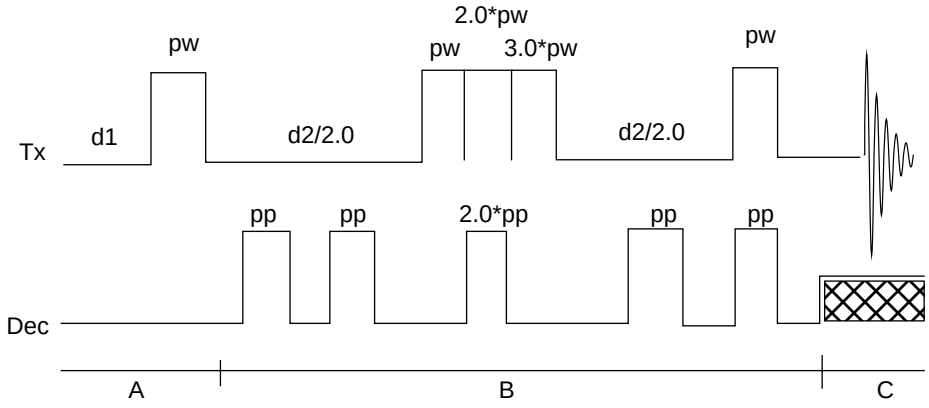


Figure 31. HETCOR Pulse Sequence

Applicability

HETCOR is available on all systems.

Parameters

`nt` should be a multiple of 4.

`d1` is 1 to 2 times the proton T_1 recommended; it must not equal 0.

`j1xh` is an average one-bond X-H coupling constant.

`jnxh` is an average 2 or 3-bond coupling constant; set it only if long-range correlations are wanted (`hmult='y'` only in this case).

`hmult='y'` for H-H multiplets; `hmult='n'` for decoupled multiplets.

`pp` is a 90° pulse on protons.

`pw` is a 90° pulse on observe nucleus.

`pplvl` is the power level for the proton pulse on the decoupler channel (systems with a linear amplifier on the decoupler rf channel).

`tpwr` is the power level for the heteronuclear pulse on the observe channel (systems with a linear amplifier on the observe rf channel).

`sw1` should equal the spectral width `sw` used to obtain proton spectrum.

`dof` should equal the value of `tof` used to obtain proton spectrum.

`presat='y'` gives presaturation pulse train; `preset='n'` for no presaturation `presat` is available on *MERCURY-VX* and *MERCURY* systems with *GLIDE* (uppercase pulse sequences only). `presat` is not active on *GEMINI 2000* systems.

`rfl1`, `rfp1`, `wp1`, `sp1` should be set to the corresponding 1D parameters from the ^{1}H spectrum.

`sw1` and `dof` should be set before typing `ga`.

Technique

The experiment can be run two different ways: (1) the standard setup optimizes for one-bond proton-carbon couplings, or (2) setting `jnxh` to the average two- or three-bond coupling constant emphasizes three-bond (and two- or four-bond) couplings.

1. A good sample to try the first time is 30% menthol in $CDCl_3$. This sample is concentrated enough that good data can be obtained in a short period of time.
2. If not in experiment 1 already, join experiment 1 by entering **`jexp1`** or by using menu buttons.
3. Set up a proton in $CDCl_3$ experiment by clicking the **H1,CDCl3** button in the Setup menu. If you are using the 30% menthol sample, set **`pw=1`**, **`gain=0`**, **`nt=1`**; otherwise, acquire the spectrum with the standard parameters.
4. Narrow the spectral window to include only the peaks of interest by placing a cursors approximately 1 ppm upfield and 1 ppm downfield of the region containing the peaks of interest and entering `movesw`.
5. Enter **`ga`** to acquire the spectrum with the new window and phase the data. This spectrum serves as a reference spectrum for the proton section of the HETCOR.
6. Enter **`jexp2`** to join experiment 2 and set up a carbon experiment by clicking the **C13,CDCl3** button in the Setup menu. Acquire a spectrum. If your are using the menthol or a similar sample, the sample is concentrated enough that one scan should be sufficient to see a carbon spectrum with good signal to noise.
7. Place a cursor upfield and a cursor downfield of the carbon signals. Narrow the spectral window by entering **`movesw`**. Set **`nt=16`** and reacquire the carbon spectrum. Phase the spectrum. This spectrum will be used as the reference for the ^{13}C dimension of the HETCOR.
8. Enter **`mp(2,3) jexp3`** to move the ^{13}C parameters from experiment 2 to experiment 3 and join experiment 3.
9. Enter **`hetcor`** to set up the HETCOR experiment and show a time estimate for performing the experiment.
10. If you are using a concentrated sample such as the menthol sample, you can modify the default parameters to produce acceptable data in a much shorter time: set

nt=16, **ni=64**, **np=1024**, **fn=1024**, **fn2=256**, and **d1=1**. Then enter **time** to check the experiment time. These parameter values should reduce the time to 20 minutes yet provide data with sufficient resolution to assign the spectrum.

11. Enter **go** to acquire the data.
12. Enter **wft2d** to Fourier transform the data. Adjust vertical scale and threshold according to the procedures outlined in "Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY," page 138.
13. Enter **plhxcor(8,1.5)** to plot HETCOR data with 8 contours spaced 1.5 levels apart.

    If you wish, try other values to create different plots. `plhxcor` can have up to four arguments: number of contours, spacing of levels, experiment number for the proton data, and experiment number for the carbon data. If the third or fourth argument is omitted, `plhxcor` assumes that the proton 1D data is in experiment 1 and that the carbon 1D data is in experiment 2. Therefore, `plhxcor` plots the contour, retrieves the 1D data, and plots them above the contour. `plhxcor` also plots expansions with the appropriate 1D plots on the side.

Potential Problems

The most common reason for failure is that the values for `pp` and `pw` are incorrect. Make sure that the 90° pulse on the decoupler, `pp` and the 90° pulse on ^{13}C, `pw` are correct before beginning the experiment.

Since both DEPT and HETCOR rely on polarization transfer between proton and carbon, a simple method to troubleshoot a HETCOR is to run DEPT on the sample. If the DEPT does not work, HETCOR probably will not work either. Once the DEPT has been properly calibrated and is working, the same values of `pw90`, `pp` (and `pplvl` if the parameter exists) should be used for the HETCOR.

Another possible problem is the presence of artifacts from pulsing too rapidly. Because of the nature of the experiment, the critical T_1s are the proton T_1s (usually 1 to 10 seconds), not the relaxation times of the carbons (which may be much longer). Make sure you set `d1` to at least the time of T_1 of the protons in the sample. A common type of artifact is an extra peak exactly between the correlations of two non-magnetically equivalent protons attached to the same carbon.

References

Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

Bax, A. *J. Magn. Reson.* **1983**, *53*, 51.

Rutar, V. *J. Magn. Reson.* **1984**, *58*, 306.

Wilde, J.; Bolton, P. *J. Magn. Reson.* **1984,** *59*, 343–346.

4.7 HETCORPS—Absolute-Value and Phase-Sensitive HETCOR

The `hetcorps` macro sets up parameters for a HETCORPS (Heteronuclear Chemical-Shift Correlation, Absolute Value and Phase Sensitive) pulse sequence.

Applicability

HETCORPS is not available on *MERCURY-VX*, *MERCURY,* or *GEMINI 2000* systems.

Parameters

`pw` is a 90° pulse on the observe nucleus.

`tpwr` is transmitter power level; only for systems with a linear amplifier on the transmitter channel.

`pp` is a proton 90° pulse on the decoupler channel.

`pplvl` is decoupler power level; only for systems with a linear amplifier on the decoupler channel; otherwise, the decoupler is turned to full-power for pulses on systems that have bilevel decoupling capability.

`dhp` is decoupler power level during acquisition.

`dpwr` is decoupler power level during acquisition for systems with linear amplifiers.

`hmult='n'` removes non-geminal proton-proton couplings in F1; `hmult='y'` preserves all proton-proton couplings in F1.

`chonly='y'` gives CH only spectrum.

`oddeven='y'` gives CH and CH_3 positive and CH_2 negative; `oddeven='n'` gives all positive. `oddeven` is irrelevant in `av` (`phase=0`) spectrum or if `chonly='y'`.

`j1xh` is a one-bond heteronuclear coupling constant.

`phase=1,2` gives hypercomplex; `phase=0` gives absolute value.

`nt=1` is the minimum for hypercomplex; `nt=2` is the minimum for absolute value. `nt` set to a multiple of 2 is recommended for hypercomplex; `nt=4` is recommended for absolute value.

Recommendations

HETCORPS cannot and should not be used for long-range correlation. For long-range correlation, use `lrhetcor.c`.

If ^{13}C parameters (after phase correcting a ^{13}C spectrum) are moved to set up HETCORPS, there should be no need to phase correct either F1 or F2.

Use gaussian windows.

4.8 HOM2DJ—Homonuclear J-resolved 2D

The `hom2dj` macro sets up parameters for a HOM2DJ pulse sequence. Absolute-value mode is required. Figure 32 is a diagram of the sequence.

Applicability

HOM2DJ is available on all systems.

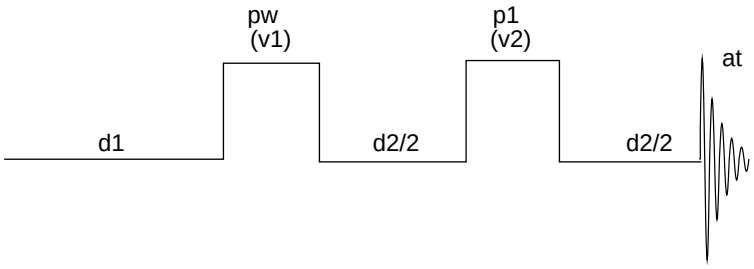


Figure 32. HOM2DJ Pulse Sequence

Parameters

`pw` is a 90° observe pulse.

`p1` is a 180° observe pulse.

`nt` is a multiple of 2 (minimum) to a multiple of 16 (maximum and recommended).

`d1` is set for 1 to 3 times T_1 (maximum); default is 1.0 seconds.

`sw1` should cover maximum multiplet pattern; the default is 50 Hz.

Technique

HOM2DJ is one of the earliest and simplest 2D experiments. Rarely used today, it has been mostly replaced by phase-sensitive COSY experiments such as DQCOSY.

1. A good sample to try at first is a 5% heptanone sample in $CDCl_3$. This sample is concentrated enough that good data can be obtained in a short time. If not there already, join experiment 1 by entering `jexp1`. Set up a proton in $CDCl_3$ by using the H1,CDCl3 button in the Setup menu. Acquire a spectrum.
2. Narrow the spectral window to include only the peaks of interest by placing a cursors about 1 ppm upfield and 1 ppm downfield of the region containing the menthol peaks and entering `movesw`. If a peak is present from residual $CHCl_3$, do not include this peak because the peak will fold in but the filters will remove most of the peak. This greatly decreases the amount of spectrum that is just noise and provides better digital resolution in the spectrum and decreased experiment time.
3. Enter **`ga`** to acquire the spectrum with the new window and phase the data.
4. Enter **`mp(1,2) jexp2`** to move the parameters from experiment 1 to experiment 2 and join experiment 2.
5. Enter **`hom2dj`** to set up the HOM2DJ experiment and display an estimate of the time required to perform the experiment.
6. You can modify the default parameters to produce acceptable data in a much shorter period of time. If you are using the heptanone sample, set **`nt=8`**, **`ni=64`**, **`np=512`**, **`fn=128`**, **`fn2=512`**, and **`d1=1`**. This provides an experiment time of 12.5 minutes yet gives sufficient resolution to assign the spectrum.
7. Enter **`go`** to acquire the data.
8. Enter **`wft2d`** to Fourier transform the data.
9. If you wish to interactively process the data, the data is in absolute-value mode and interactive processing is the same as for COSY (see page 138).

10. After transformation, the data is tilted along an angle of 45°. Enter **`rotate(45.0)`** to rotate the data.
11. Enter **`foldj`** to symmetrize the data.
12. Display the data contours in the manner described in "Absolute-Value COSY, RELAY-COSY, Double RELAY-COSY," page 138.
13. Plot the data using the procedure described in "HET2DJ—Heteronuclear 2D-J," page 145.

Potential Problems

The HOM2DJ experiment is very forgiving and usually works (to a greater or lesser extent) with slightly incorrect `pw` and `p1` values. The most common reason for failure of the experiment is that the values for `pw` are incorrect. Make sure that the 90° pulse is correct before beginning the experiment.

A major problem, inherent to the experiment, is that HOM2DJ results in phase twisted lineshapes that can make interpretation difficult in crowded regions and causes artifacts (which show up as extra peaks) due to strong coupling.

References

W. Aue, J. Karhan, and R. Ernst, *J. Chem. Phys.* **64**:4226 (1976).

K. Nagayama, O, Backmann, K. Wuthrich, and R. Ernst, *J. Magn. Reson.* **31**:133 (1978).

4.9 INADEQUATE—Double-Quantum Transfer Experiment

The `inadqt` macro sets up parameters for an INADEQUATE (Incredible Natural Abundance Double-Quantum Transfer Experiment) pulse sequence. Figure 33 shows a diagram of the sequence.

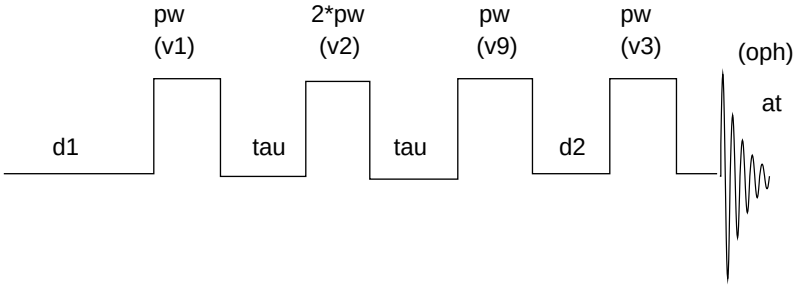


Figure 33. INADEQUATE Pulse Sequence

Applicability

INADEQUATE is available on all systems except *GEMINI 2000*.

Parameters

`pw` is a 90° pulse on observed nucleus (e.g., carbons) at power equal to `tpwr`.

`tau` is set to 1/(4*J_{CC}).

`jcc` is the average scalar coupling constant between the two heteronuclei (usually one-bond constants).

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`dmf` sets the modulation frequency for WALTZ-16 broadband decoupling of protons at power equal to `dhp` or `dpwr` throughout the pulse sequence.

`nt` is a multiple of 8 (minimum: `phase=0`), a multiple of 128 (maximum: `phase=0`), a multiple of 4 (minimum: `phase=1,2` or `phase=3`), or a multiple of 64 (maximum: `phase=1,2` or `phase=3`).

`dm` is set to `'y'`.

`dmm` is set to `'w'`.

`hs` is set to `'yn'`.

`hst` is set to 0.01.

`phase=0` for 2D absolute-value data, `phase=1,2` for 2D hypercomplex data (States-Haberkorn method), or `phase=3` for 2D TPPI data. `phase=1,2` is the suggested value. For `phase=3`, remember that `sw1` must be set to *twice* the desired value.

Technique

Set up the sequence from an existing ^{13}C spectra.

If data was acquired with `phase=0`, process with `wft2d`, but if data was acquired with `phase=1,2` or `phase=3`, process with `wft2da`. If phase-sensitive data without f_1 quadrature are desired, set `phase=1` and process with `wft2da`.

This experiment must be performed non-spinning and with VT regulation. To obtain reasonable results within 24 hours, a one-transient ^{13}C spectrum should present a signal-to-noise ratio of at least 25:1 (1.5 sec. recycle time, 24 hours total acquisition time, 128 total increments). For 1D spectra, set `phase=1` for maximum sensitivity.

4.10 MQCOSY—Multiple-Quantum Filtered COSY

The `mqcosy<(level)>` macro sets up parameters for a MQCOSY pulse sequence, where the optional argument `level` is the desired quantum level of filtration (e.g., `mqcosy(3)`). Figure 34 is a diagram of the sequence.

Applicability

MQCOSY is available on all systems except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*.

Parameters

`pw` is the 90° pulse on the observed nucleus.

`d1` is the relaxation delay (1 to 3 times the value of t_1).

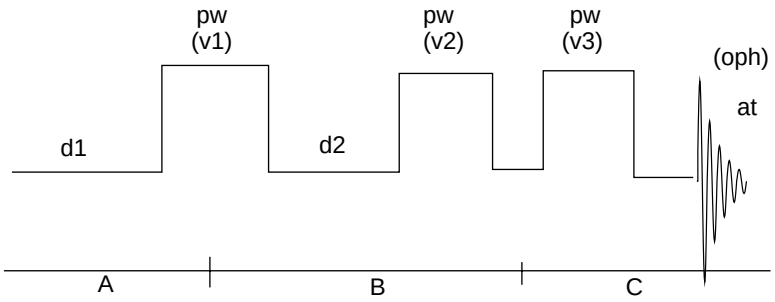


Figure 34. MQCOSY Pulse Sequence

`sw` is the spectral width (usually `sw1=sw`, except for `phase=3`).

`qlvl` is the quantum level of filtering desired in the experiment.

`phase=1,2` for 2D hypercomplex data (States-Haberkorn method) or `phase=3`: 2D TPPI data. `phase=1,2` is suggested. Note: For `phase=3`, remember that `sw1=2*sw` (for homonuclear experiments) or that `sw1` must be set to *twice* the desired value (for heteronuclear experiments).

`sspul='y'` activates a homospoil-90-homospoil sequence that precedes `d1`; this is used to achieve a less oscillatory steady-state for 2D experiments where the recycle time is shorter than t_1.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm` but does depend on `dmm` and is activated as a part of `d1` if `presat` is greater than 0.

`nt` is a multiple of `qlvl` (minimum) or a multiple of `2*qlvl` (recommended).

Technique

Set up the MQCOSY in the same manner that you set up DQCOSY. Set the parameter `glvl` to the desired multiple-quantum level.

To process, enter `wft2da` or use the menu buttons to perform full processing.

To interactively process, use the technique given in "DQCOSY—Double-Quantum Filtered COSY," page 143.

References

Piantini, U.; Sorenson, O.; Ernst, R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.

Rance, M. et al. *Biochem. Biophys. Res. Comm*. **1983,** *117*, 479–485.

4.11 NOESY—Nuclear Overhauser Effect Spectroscopy

The `noesy` macro sets up parameters for a NOESY Laboratory Frame Overhauser or 2D Exchange pulse sequence. It can be performed in either a phase-sensitive or absolute-value mode. Either TPPI or the hypercomplex method can be used to achieve f_1 quadrature in a phase-sensitive presentation. No attempt is made to suppress J-cross peaks. Figure 35 is a diagram of the sequence.

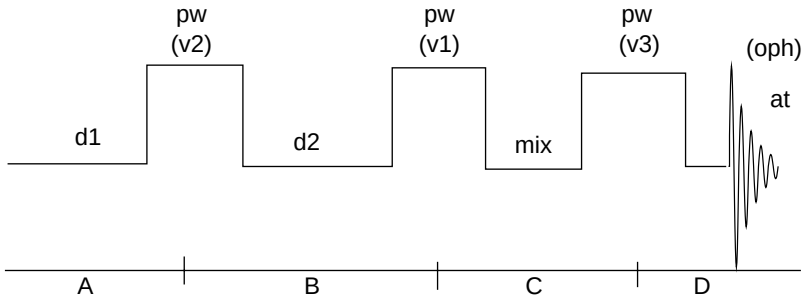


Figure 35. NOESY Pulse Sequence

Applicability

NOESY is available on all systems.

Parameters

`pw` is a 90° pulse on the observed nucleus (power = `tpwr`).

`d1` is the relaxation delay (1 to 3 times the value of T_1).

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (`sw1=sw` usually, except for `phase=3`).

`ni` is the number of t_1 increments (set up by default so that `sw1/ni=12`).

`mix` is the mixing time for magnetization exchange.

`phase=1,2` is the suggested value; use `phase=0` for P-type peak selection for `av` display; `phase=1,2` for 2D hypercomplex data (States-Haberkorn method); `phase=3` for 2D TPPI data.

`sspul='y'` activates a homospoil-90-homospoil sequence that precedes `d1`; this achieves a less oscillatory steady-state for 2D experiments where recycle time is shorter than T_1. `sspul` has no effect on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm` but does depend on `dmm` and is activated as a part of `d1` if `presat` is greater than 0. `presat` is available on *MERCURY-VX* and *MERCURY*

systems with *GLIDE* (uppercase pulse sequences only). `presat` is not active on *GEMINI 2000* systems.

`dm='nnnn'` is no decoupler presaturation (unless `presat>0`);
`dm='nynn'`: decoupler presaturation during evolution period;
`dm='nnyn'`: decoupler presaturation during mixing period;
`dm='nnny'`: homonuclear decoupling during t2;
`dm='nyyn'` is recommended when solvent saturation is needed.

`nt` is a multiple of 8 (minimum) for `phase=1,2` or `phase=3`; `nt` is a multiple of 16 (minimum) for `phase=0`.

Phase Cycling

`v1` is the phase for the second `pw` pulse.
`v2` is the phase for the first `pw` pulse.
`v3` is the phase for the third `pw` pulse.
`oph` is the phase for receiver.

```
v1  =  x  x  x  x  x  x  x  x  y  y  y  y  y  y  y  y
      -x -x -x -x -x -x -x -x -y -y -y -y -y -y -y -y
v2  =  x -x  x -x  x -x  x -x  y -y  y -y  y -y  y -y
v3  =  x  x  y  y -x -x -y -y  y  y -x -x -y -y  x  x
oph =  x -x  y -y -x  x -y  y  y -y -x  x -y  y  x -x
      -x  x -y  y  x -x  y -y -y  y  x -x  y -y -x  x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add 90*(`ix` – 1)° to `v1`, where `ix` is the increment counter. For `phase=0`, the P-type selection subcycle is added in after the first eight steps in the phase cycle.

For `phase=3`, remember that `sw1` equals `2*sw` (for homonuclear experiments) or that `sw1` must be set to *twice* the desired value (for heteronuclear experiments).

If `mix` is arrayed, then `phase=3` is necessary in order to be able to process the data with the `wft2dac` macro.

Technique

Because NOESY is a phase-sensitive 2D experiment, NOESY spectra may need to be phased before good results are obtained.

1. A good sample to try the first time would be a 5% sucrose sample in D_2O. This sample is concentrated enough that good data can be obtained in a relatively short time. If not there already, join experiment 1 by entering **`jexp1`** or by using menu buttons. Set up a proton in D_2O by clicking on the following buttons from the Main Menu: **Setup** > **Nucleus, Solvent** > **H1** > **D2O**. Acquire a spectrum.
2. Narrow the spectral window to include only the peaks of interest by placing a cursors about 1 ppm upfield and 1 ppm downfield of the region containing the peaks and enter `movesw`. This greatly decreases the amount of spectrum that is just noise and provides better digital resolution in the spectrum and decreased experiment time. Generally you want to obtain better digital resolution in phase-sensitive experiments than in absolute-value experiments.
3. Enter **`go`** to acquire the spectrum with the new window and phase the data. This spectrum will serve as a reference spectrum for the NOESY.
4. Enter **`mp(1,4) jexp4`** to move the parameters from experiment 1 to experiment 4 and join experiment 4.

5. Enter **noesy** to set up the NOESY experiment and display an estimate of the time required to perform the experiment.
6. If you are running the sucrose sample, set **nt=16**, **ni=128**, **np=1024**, **fn=1024**, **fn2=1024**, and **d1=2**. The experiment takes about three hours.
7. Enter **go** to acquire the data.
8. Enter **wft(1)** to Fourier transform the first increment of data. The spectrum appears inverted, but this is normal. When the spectra are Fourier transformed, the diagonal is below the plane of the 2D, and NOE crosspeaks due to positive NOEs appear positive, and crosspeaks due to negative NOEs are negative. Phase the spectrum, leaving it properly phased but inverted. This sets the phase for the f_2 dimension of the 2D.
9. Enter **wti** to start interactive weighting. NOESY data is phase-sensitive and usually processed using Gaussian weighting, the default weighting function calculated by the setup macro. Adjust the Gaussian weighting so that data decays to zero before the end of the window. Adjusting the weighting function on the first increment of the data sets the weighting function in the 2D time dimension t_2.
10. Enter **wft1da** to Fourier transform the t_2 dimension. A contour map of f_2,t_1 is displayed, showing individual interferograms. Click on trace and choose a trace through one of the interferograms. Enter **wti** to bring up interactive weighting of the interferogram. Adjust the weighting function as before.
11. Enter **wft2da** to complete the Fourier transformation.
12. The f_1 dimension may now need phasing. To phase f_1, click on trace and select a trace at the top (upfield) section of the 2D. Enter **ds** to display the trace, and phase normally using the parameter **rp**.
13. Enter **dconi** to redisplay the contour map with a new `rp`. Click on trace again, and select a trace at the bottom (downfield) section of the 2D. Enter **ds** and click on phase. Move the cursor upfield and click. *Do not adjust the phase at this point.* Clicking at this point sets `rp` and retains the `rp` value obtained previously. Move downfield and click. Adjust phase normally. This adjusts `lp`. Enter **dconi** to redisplay the properly phased 2D.
14. Enter **plcosy** to plot the data. The `plcosy` macro is general and plots all homonuclear correlated data.

Potential Problems

Unlike the COSY experiment, obtaining good NOESY spectra requires proper values of 90° pulse width and a consideration of delay times. Make sure that the 90° pulse is correct before beginning the experiment. If 90° pulse is incorrect, many “COSY type” (i.e. antiphase cross-peaks) appear, complicating the analysis. In small molecules, some “COSY-type” cross-peaks may be unavoidable even when everything is carefully calibrated. Fortunately these can be easily distinguished because of their antiphase nature, i.e., the cross-peaks have both positive and negative components but true NOE peaks are pure absorptive.

Another possible problem is the presence of artifacts due to pulsing too rapidly. Make sure you set `d1` to at least 1 to 3 times the T_1 of the protons in the sample. The NOESY experiment must be interpreted more carefully than the COSY experiment because cross-pulses arise from COSY interaction as well as dipolar interaction.

Reference

States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

4.12 ROESY—Rotating Frame Overhauser Effect Spectroscopy

The `roesy<(ratio)>` macro sets up parameters for a ROESY pulse sequence, where the optional argument `ratio` is the desired value of the parameter `ratio` used in the sequence (`ratio` is not used in the ROESY sequence provided with *MERCURY-VX* and *MERCURY*). Either time-shared or a continuous-wave spin lock can be used. Compensation for off-resonance effects is performed. Figure 36 shows the sequence.

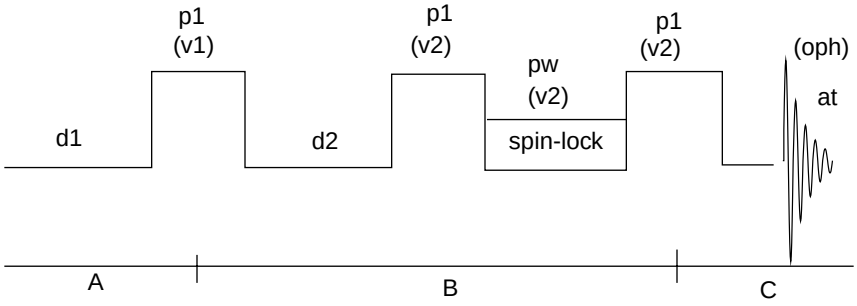


Figure 36. ROESY Pulse Sequence

Applicability

ROESY is available on all systems except *GEMINI 2000* systems; however, the ROESY pulse sequence on *MERCURY-VX* and *MERCURY* systems are slightly different from other systems. *MERCURY-VX* and *MERCURY* use a simple CW spinlock, and there are only two parameters that determine the spinlock: `slpwr` is the power for the CW spinlock and `mix` is the time. `sspul` and `ratio` do not exist in the *MERCURY-VX* and *MERCURY* parameter sets. `presat` is available on *MERCURY-VX* and *MERCURY* systems with *GLIDE* (uppercase pulse sequences only).

Parameters

`ratio` is used to adjust the effective spin-lock field strength provided by the transmitter during the spin-lock period. As `ratio` is increased, the effective spin-lock field strength is decreased. If `ratio` is set too short, TOCSY peaks begin to appear regardless of the value for `pw`. If `ratio` is set too long, the protons are not spin locked in the rotating frame and therefore do not exchange magnetization via cross relaxation in the rotating frame. The parameter `ratio` should be increased until the protons of interest begin to show sufficient amplitude and phase stability.

`mix` (the mixing time) is the length of the spin-lock, which can be a continuous wave (`ratio=0`) or pulsed (`ratio` is not 0). If pulsed, a series of pulses of length `pw` and delays of length `ratio*pw` are applied to form the spin-lock period. The effective spin-lock field strength (B(spinlock)) can be calculated by the relation B (spin-lock) = (x/(360*`pw`)/(`ratio` + 1), where x is the flip angle of the `pw` pulse.

Note that to achieve resonance offset compensation, this sequence employs two hard 90° pulses on both sides of the spin-lock. If resonance offset compensation is not desired, set `rocomp='y'`.

`p1` is a 90° pulse on the observed nucleus (power is `p1lvl`).

`pw` is an *x*° (default is 30°) pulse on the observed nucleus (power is `tpwr`); note that `pw` and `ratio` are used to define the B_1 field for the spin-lock. A typical value of `ratio` is 10 for a 30° `pw` pulse.

`d1` is the relaxation delay (1 to 3 times the value of T_1).

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (`sw1=sw` usually, except for `phase=3`).

`ni` is the number of t_1 increments (set up so that `sw1/ni` equals 12).

`phase=1,2` (suggested value), `phase=1,2` for 2D hypercomplex data (States-Haberkorn method), or `phase=3` for 2D TPPI data.

`sspul='y'` activates a homospoil-90-homospoil sequence that precedes `d1`; this is used to achieve a less oscillatory steady-state for 2D experiments where the recycle time is shorter than t_1.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm` but does depend on `dmm` and is activated as a part of `d1` if `presat` is greater than 0.

`nt` is the multiple of 4 (`phase=1,2` or `phase=3`) (minimum).

Phase Cycling

`v1` is the phase for `pw` pulse.
`v2` is the phase for the spin lock.
`oph` is the phase for receiver.

```
v1  =  y -y -x  x
v2  =  x  x  y  y -x -x -y -y
oph =  y -y -x  x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add 90*(`ix-1`)° to `v1`, where `ix` is the increment counter.

For `phase=3`, remember that `sw1` equals `2*sw` (for homonuclear experiments) or that `sw1` must be set to *twice* the desired value (for heteronuclear experiments).

Technique

To set up and acquire, use the technique given in “NOESY—Nuclear Overhauser Effect Spectroscopy,” page 155.

To process for normal `phase=1,2`, use the technique (except spectra are *not* inverted) given in “NOESY—Nuclear Overhauser Effect Spectroscopy,” page 155. If `mix` is arrayed, `phase=3` is necessary to be able to process the data with the `wft2dac` macro.

Reference

Kessler, et al. *J. Am. Chem. Soc*. **1987**, January.

4.13 TNCOSYPS—COSYPS with Water Suppression

The `tncosyps` macro sets up parameters for homonuclear correlation (phase-sensitive version) that uses transmitter solvent saturation.

Applicability

TNCOSYP requires a system with a linear amplifier on the observe channel and a T/R switch. It is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 141 for a discussion of COSYPS.

Parameters

`satmode` determines when the saturation happens. `satmode` should be set analogously to `dm`, (e.g., `satmode='yyn'` or `satmode='ynn'`).

`satpwr` is the power level during saturation period(s).

`satdly` is the length of presaturation period (saturation may also occur in `d2` as determined by `satmode`).

`sspul='y'` selects for `trim(x)-trim(y)` sequence at start of pulse sequence (recommended)

4.14 TNDQCOSY—DQCOSY with Water Suppression

The `tndqcosy` macro sets up parameters for a DQCOSY experiment with transmitter solvent saturation only. The experiment assumes an on-resonance solvent (`tof` is at solvent position).

Applicability

DQCOSY requires a system with a linear amplifier on the observe channel and a T/R switch. It is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 143 for a discussion of DQCOSY.

Parameters

`satmode` determines when the saturation happens. `satmode` should be set analogously to `dm` (e.g., `satmode='yyn'` or `satmode='ynn'`).

`satpwr` is the power level during saturation period(s).

`satdly` is the length of presaturation period (saturation may also occur in `d2` as determined by `satmode`).

`sspul='y'` selects for `trim(x)-trim(y)` sequence at start of pulse sequence (recommended)

4.15 TNMQCOSY—MQCOSY with Water Suppression

The `tnmqcosy` macro sets up parameters for a multiple-quantum filtered COSY experiment with transmitter saturation.

Applicability

TNMQCOSY uses the hardware digital phaseshifter for the transmitter with direct synthesis rf. It uses the software small-angle phaseshifter for the transmitter with old-style rf. It is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 153 for a discussion of MQCOSY.

Parameters

`pw` is a 90° excitation pulse (at power level `tpwr`).

`phase=1,2` for hypercomplex phase-sensitive experiment or `phase=3` for TPPI phase-sensitive experiment. If `phase=3`, remember that `sw1` must be set to twice the desired value.

`satmode='ynn'` saturates during relaxation delay.

`satdly` is the saturation time.

`satpwr` is the saturation power.

`sspul='y'` selects for `trim(x)-trim(y)` sequence at start of pulse sequence; `sspul='n'` selects a normal MQCOSY experiment.

Note: Earlier versions included the following sequence at the beginning of the pulse sequence: *homospoil - 90° pulse - homospoil*. This was used to eliminate both the DQ-like artifacts in the 2D spectrum and the oscillatory nature of the steady-state. This inclusion was selected if `sspul='y'`.

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`qlvl` selects the quantum order for filtering (2, 3, etc.).

`nt` minimum is a multiple of `2*qlvl`; `nt` maximum is a multiple of `8*qlvl`.

References

Piantini, U.; Sorenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* ***1982***, ***104***, 6800–6801.

Rance, M.; et al. *BBRC*, **1983**, ***117***, 479-485.

4.16 TNNOESY—NOESY with Water Suppression

The `tnnoesy` macro sets up parameters for a 2D cross-relaxation experiment with transmitter saturation. It assumes an on-resonance solvent (`tof` is at solvent position)

Applicability

TNNOESY requires a system with a linear amplifier on the observe channel and a T/R switch. It is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 155 for a discussion of NOESY.

Parameters

`satmode` determines when the saturation happens. `satmode` should be set analogously to `dm` (e.g., `satmode='yyyn'` or `satmode='ynyn'`).

`satpwr` is the power level during saturation period(s).

`satdly` is the length of presaturation period (saturation may also occur in *d2* and *mix* as determined by `satmode`).

`sspul='y'` selects for `trim(x)-trim(y)` sequence at start of pulse sequence.

4.17 TNROESY—ROESY with Water Suppression

The `tnroesy` macro sets up parameters for a rotating frame NOE experiment. Observe transmitter should be set at solvent position. Saturation, spin lock, and pulses all use the observe transmitter.

Applicability

TNROESY is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 158 for a discussion of ROESY.

Parameters

`p1` is the 90° pulse on protons (power level at `p1lvl`).

`pw` is a small (30°) pulse on protons (active only if `ratio` is greater than 0).
If `pw=0`, `pw` is set to `p1/3`.

`p1lvl` is the power level for the `p1` pulse.

`tpwr` is the power level for the spin lock pulse(s).

A cw lock is used if `ratio` is zero.

`phase=1,2` gives f_1 quadrature by the hypercomplex method (uses f_1 axial peak displacement). `phase=3` gives f_1 quadrature by the TPPI method.

`mix` is the mixing time.

`sspul='y'` selects for `trim(x)-trim(y)` sequence at start of pulse sequence.

`rocomp='n'` sets no resonance offset compensation
`rocomp='y'` sets resonance offset compensation (recommended).

`satmode` is the saturation mode. Use analogously to `dm`, for example,
`satmode='nnn'`, `satmode='ynn'`, or `satmode='yyn'` (recommended).

`satdly` is the length of saturation during relaxation delay.

`satpwr` is the power level for solvent saturation.

`nt` minimum is a multiple of 2; `nt` maximum is multiple of 8 (recommended)

`d2corr` is an empirical correction, in µs, of `d2` (dependent on effective field of spin lock, i.e., `tpwr` and `ratio`). It can be determined from the `lp1` and `sw1` values from a properly phased spectrum by `d2corr=(lp1*1e6)/(360*sw1)`. Note that the `d2corr` seems to be dependent on `sw1`; however, it is independent of `sw1` since changes in `sw1` result in corresponding changes in `lp1` so that their ratio is constant.

Technique

The following procedure finds `d2corr` so that `lp1` is 0, giving better baselines in f_1:

1. Run a TNROESY experiment with **`d2corr`** set either at 0 or at a value found previously. (`nt` and `ni` can be smaller, and the spectrum can be transformed early to do step 2.)
2. Phase the resulting spectrum in f_1. Determine `lp1` and calculate `d2corr` from the relationship `d2corr=(lp1*1e6)/(360*sw1)`.
3. Add this value to the value of `d2corr` used in step 1.
4. Rerun the experiment. **`lp1`** should be close to zero.
5. Note this value for any future experiment with the same value of `tpwr` and `ratio`.

Use of any method to make `lp1=0` will result in a dc offset of F_1 slices. This should be removed by `dc2d('f1')` after the 2D transform. Enough noise should be left on the edges (in F_1) to permit this dc correction.

4.18 TNTOCSY—TOCSY with Water Suppression

The `tntocsy` macro sets up parameters for total correlation spectroscopy (also known as HOHAHA) using transmitter presaturation. It features “clean” TOCSY with optional windowing and MLEV16 + 60° spin lock. The transmitter must be positioned at the solvent frequency.

Applicability

TNTOCSY requires a T/R switch, and linear amplifiers and computer-controlled attenuators on the observe channel. It is not available on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems. See page 164 for a discussion of TOCSY.

Parameters

`pw` is a 90° pulse during `mlev` periods, at power level `tpwr`.

`p1` is a 90° excitation pulse, at power `p1lvl`.

`window` is a clean-TOCSY window, in µs.

`satdly` is the length of presaturation.

`satmode='yn'` for `presat` control during relaxation delay only, `satmode='yy'` for `presat` control during both the “relaxation delay” and `d2`.

`phase=1,2` for hypercomplex phase-sensitive f_1 quadrature, or `phase=3` for TPPI phase-sensitive f_1 quadrature.

`sspul='y'` gives the `trim(x)-trim(y)` sequence at beginning of `d1` delay; `sspul='n'` gives the normal `d1` delay.

`trim` is a spinlock trim pulse time (0.002 recommended).

`mix` is the mixing time (can be arrayed).

`nt` minimum is a multiple of 2, `nt` maximum is a multiple of 8 (recommended).

References

Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, ***65***, 355.

Levitt, M.; Freeman, R.; Frenkiel, T. *J. Magn. Reson.* **1982**, ***47***, 328.

4.19 TOCSY—Total Correlation Spectroscopy

The `tocsy` macro sets up parameters for the TOCSY pulse sequence. Figure 37 is a diagram of this sequence. TOCSY is also known as the Homonuclear Hartmann-Hahn experiment (HOHAHA).

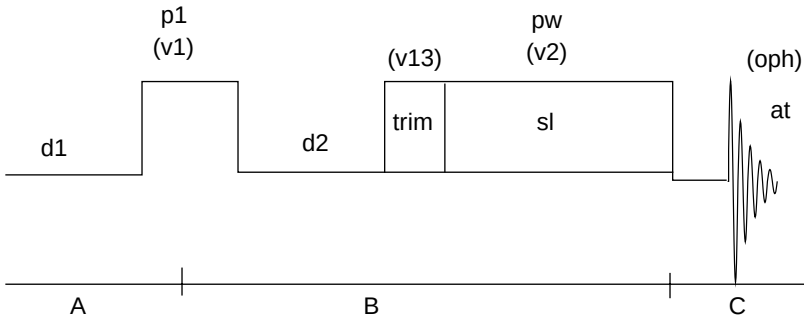


Figure 37. TOCSY Pulse Sequence

Applicability

TOCSY requires systems having linear amplifiers with computer-controlled attenuators on the observe transmitter channel. It is not available on the *GEMINI 2000*.

Parameters

`p1` is the 90° pulse on the observed nucleus (at power equals `p1lvl`)

`pw` is the 90° pulse on the observed nucleus (at power equals `tpwr`); note that `tpwr` is used to define the B_1 field for both the trim (`trim`) pulses and the MLEV-16 spin lock. The B_1 field at `tpwr` should be on the order of 15 ppm (in Hz).

`trim` is the trim pulse time (CW transmitter irradiation).

`sl` is MLEV-16 spin lock. `mix` is the duration of the spin lock, recommended mixing time is 30 to 80 ms. `mix` can be arrayed.

`d1` is a relaxation delay (1 to 3 times the value of t_1).

`phase=1,2` (suggested value) for 2D hypercomplex data (States-Haberkorn method); `phase=3` for 2D TPPI data. For `phase=3`, remember that `sw1=2*sw` (for homonuclear

experiments) or that sw1 must be set to *twice* the desired value (for heteronuclear experiments).

sspul='y' activates a homospoil-90-homospoil sequence that precedes d1*;* This is used to achieve a less oscillatory steady-state for 2D experiments where the recycle time is shorter than t_1.

ss is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if ss is positive, ss steady-state pulses are applied on the first increment only; if ss is negative, –ss transients are applied at the start of each increment.

presat is decoupler presaturation period using a decoupler power specified by dhp or dpwr. presat does not depend on dm but does depend on dmm and is activated as a part of d1 if presat is greater than 0. presat is available on *MERCURY-VX* and *MERCURY* systems with *GLIDE* (uppercase pulse sequences only).

nt is a multiple of 4 (minimum, phase=1,2 or phase=3).

Phase Cycling

v1 is the phase for p1 pulse
v13 is the phase for trim pulses
v2 is the master phase for the MLEV-17 spin lock
oph is a phase for receiver

```
v1  =  y -y -x  x
v13 =  x  x  y  y
v2  =  y  y -x -x -y -y  x  x
oph =  y -y -x  x  y -y -x  x
```

These phases are for phase=1. For phase=2, add 90° to v1. For phase=3, add 90*(ix–1)° to v1, where ix is the increment counter.

Technique

To set up and acquire, use the technique given in "NOESY—Nuclear Overhauser Effect Spectroscopy," page 155. Set mix to 0.060.

To process simple TOCSY, use the technique (except spectra in the first increment are *not* inverted) given in "NOESY—Nuclear Overhauser Effect Spectroscopy," page 155.

For a series of TOCSY experiments acquired in an interleaved fashion, each with a different mixing time, process the data with the wft2dac(mult1,mult2,...) command. Up to eight interleaved experiments can be processed, which means that mix can be given up to eight different values. Note that if mix is arrayed, phase=3 is necessary in order to be able to process the data with the wft2dac macro.

References

Levitt, M.; Freeman, R.; Frenkiel, T. *J. Magn. Reson.* **1982**, ***47***, 328.

Bax, A.; Davis, D. *J. Magn. Reson.***1985**, *65*, 355.

Related Macros

The following macro are used with TOCSY (with multiple mixing times):

- `ft1dac` and `ft2dac` combine 2D FID matrices within the 2D Fourier transform framework.
- `wft1dac` and `wft2dac` also combine 2D FID matrices within the 2D Fourier transform framework but with weighting added.

Refer to description of these macros in the *VNMR Command and Parameter Reference* for further information, including the arguments used with each macro.

4.20 TROESY—Transverse ROESY

The `troesy` macro sets up parameters for the TROESY pulse sequence, a transverse cross-relation experiment in a rotating frame.

Applicability

TROESY is not available on *MERCURY-VX*, *MERCURY*, or *GEMINI 2000* systems.

Parameters

TROESY uses typical ROESY parameters. Note that `ratio` is not a parameter.

Set `slpwr` to the spinlock desired region.

`slpw` is the 90° pulse width at `slpwr`.

Reference

Shaka, et. al. *J. Am. Chem. Soc*. **1992**, ***114***, 3157.

4.21 HCCHTOCSY Pulse Sequence

The `hcchtocsy` macro sets up parameters for the HCCHTOCSY pulse sequence, used for sidechain assignments in fully ^{13}C-enriched biopolymer.

Applicability

Not supplied with *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`coshape` is the decoupler pattern for CO decoupling.

`fhfdwt1` is a flag to indicate half dwell start in t_1.

`fhfdwt2` is a flag to indicate half dwell start in t_2.

`tof` is set in center of proton spectrum or on H_2O frequency if in H_2O.

`dof` is set in center of the ^{13}C aliphatic region.

`satfrq` is the presaturation frequency.

`pwca` is the 90° pulse width for the ^{13}C nucleus.

`pwco` is the 90° pulse width for the ^{13}C carbonyl decoupling.

`satpwr` is the low-level ^{1}H transmitter power for `presat`.

`pwcalvl` is the power level for ^{13}C pulses.

`pwcolvl` is the power level for C=O decoupling pulse.

`jch` is coupling for C-C (set to 40 Hz).

`ncyc` is the number of cycles through the `dipsi` loop.

`trim` is the trim pulse length, in seconds.

`dipsipwr` is the power level for ^{13}C spin lock.

`p1` is the 90° pulse width for `dipsi`.

`cycletime` is an informational parameter (do not enter).

Technique

Optional ^{13}C decoupling of carbonyl carbons during t2 uses a 180° pulse done through a shaped SLP (phase-ramped) pulse. The ^{13}C transmitter is normally in aliphatic region (do not try for both aliphatic and aromatic `hcchtocsy` because the bandwidth is too large for good spinlock). The pulse is of length `pwco` at power `pwcolvl` and of name `coshape`.

1. Determine the length, nature and power for this 180 in an on-resonance calibration experiment.
2. Prepare this pulse by preparing a “template” pulse that has 5 times as many steps as width, in µs.
3. Convolute this to prepare the SLP pulse. For example, if `pwcolvl` is 38, a sinc pulse does a 180° pulse in 250 µs when on-resonance; therefore, for the most accuracy you would need a `sinc.RF` file of 250*5 steps from `shapelib`.

   For rectangular pulses, you can run **`makehard`**`(number_steps)` to do this. For complex shapes, you can generate it out of **`pulsetool`** or by a separate program.

   If the sinc pulse has the name `sinc1250.RF`, and the distance from the ^{13}C transmitter is, say, 15000 Hz, enter

   `convolute('sinc1250','co180_1250us_+15000',250,15000)`

   to prepare the new shape. This gives you a shape that is used by `decshaped_pulse` to do a 180 on the carbonyl region when `dof` is positioned in the aliphatic region. Use `pulsetool` to verify that your shape is correct.

   It is not necessary to do carbonyl decoupling. The only visible effect is CC splitting in f_2 for alpha carbons. Setting `pwco=0` is not desirable.
4. Set **`dm='nny'`** to do ^{13}C decoupling during acquisition. One `dipsi-3` cycle is `217.33*p1`.

   For example, if `p1` is 36 µs, a single `dipsi` cycle is 7.8 ms.
5. Set **`phase=1,2`** and **`phase2=1,2`** for hypercomplex in t_1 and t_2.

   Typical acquisition times are 28 ms for t_1, 10 ms for t_2, and 47 ms for t_3, with 128 complex points for t_1, 32 complex for t_2, and 512 real for t_3.

4.22 HMQCTOCSY Pulse Sequence

The `hmqctocsy` macro sets up parameters for HMQCTOCSY pulse sequence, with an option to null or invert the direct responses.

Applicability

Not supplied with *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`p1` is the 90° pulse during the clean-TOCSY period.

`p1lvl` is the power level for pulse `p1`.

`window` is the off-time for clean-TOCSY. If `p1=25`, `window=50` (in µs) is typical.

`pw` is the ^{1}H 90° pulse during the pulse sequence outside of the `mlev` period.

`tpwr` is the power level for `pw`.

`mix` and `trim` are the isotropic mixing period, 0.020 and 0.002 are typical values.

`mult` controls the direct responses:
- `mult=0` gives a normal HMQCTOCSY (direct and relayed in-phase).
- `mult=1` nulls the direct responses. `dm='nnnn'` is best for this.
- `mult=2` inverts the direct responses.

Technique

Remember to enter **`dps`** to check things before entering **`go`**.

4.23 HMQC-TOCSY 3D Pulse Sequence

The `hmqctocsy` macro sets up parameters for HMQC-TOCSY 3D pulse sequence with presaturation option, written in hypercomplex phase-sensitive mode only. Figure 38 is a diagram of the sequence.

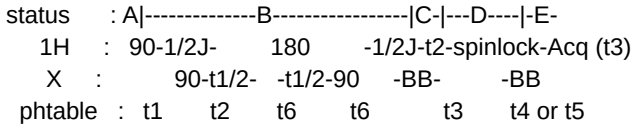


Figure 38. HMQC-TOCSY 3D Pulse Sequence

Applicability

Not supplied with *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`d2` is first evolution time.

`d3` is second evolution time.

`mix` is the TOCSY mixing time.

`pwxlvl` is the power level for X pulses.

`pwx` is a 90° X pulse.

`j1xh` is a X-H coupling constant.

`dpwr` is the power level for X decoupling.

`tpwr` is the power level for H pulses.

`pw` is a 90° H hard pulse.

`slpwr` is the power level for spinlock.

`slpw` is a 90° H pulse for mlev17.

`trim` is a trim pulse preceding mlev17.

`phase=1,2` gives hypercomplex (t1) acquisition; `ni` is number of t1 increments.
`phase2=1,2` gives hypercomplex (t2) acquisition; `ni2` is number of t2 increments

`satflg='y'` is presaturation during `satdly`.

`satfrq` is the presaturation frequency.

`satdly` is the saturation time during the relaxation period.

`satpwr` is the saturation power for all periods of presaturation with the transmitter.

`wdwfctr` is multiplication "window" factor of `slpw`.

`nullflg` is TANGO `nullflg` flag for protons not attached to X.

`hmqcflg='n'` turns off HMQC part of the sequence.

Technique

For F1 x F3, use **`wft2d(1,0,0,0,0,0,-1,0)`** (i.e., `wft2da`).

For F2 x F3, use **`wft2d(1,0,0,0,0,0,-1,0)`** if `hmqcflg='n'` (i.e., `wft2da`) or **`wft2d(1,0,0,0,0,0,1,0)`** if `hmqcflg='y'`.

For 3D processing: create 3D coefficients using **`make3dcoef`** macro:
`f1coef='1 0 0 0 0 0 -1 0'` and **`f2coef='1 0 0 0 0 0 1 0'`**.

4.24 HSQC-TOCSY 3D Pulse Sequence

The `hsqctoxySE` macro sets up parameters for HSQC-TOCSY 3D pulse sequence with many features: HSQC and TOCSY are each "sensitivity enhanced" as per Rance et. al. The sequence features a dipsi or mlev-17 spinlock option (`dipsiflg` flag). F1 dimension can be band selected with shaped pulse. (Use of shaped pulse introduces a small phase error along F1. `rp1` and `lp1` values can be obtained by examining first `np` x `ni` plane) The sequence has a gradient to kill unwanted signals during H-X INEPT. Water presaturation can be on- or off- resonance.

Applicability

Not supplied with *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`phase=1,2,3,4` and `phase2=1,2,3,4.`

`dm='nnyny'` (on during t2 and t3).

`f2coef='1 0 0 1 1 0 0 -1 0 1 -1 0 0 -1 -1 0'` and
`f1coef='1 0 0 1 1 0 0 -1 0 1 -1 0 0 -1 -1 0'.`

`f1coef` and `f2coef` are processing parameters used by the `make3dcoef` macro.

Chapter 5. Indirect Detection Experiments

Sections in this chapter:

- 5.1 “Requirements for Indirect Detection Experiments,” this page
- 5.2 “The Basic HMQC Experiment,” page 177
- 5.3 “Phase-Sensitive Aspects of the Sequence,” page 181
- 5.4 “Cancellation Efficiency,” page 181
- 5.5 “Pros and Cons of Decoupling,” page 182
- 5.6 “Specifications Testing,” page 183
- 5.7 “Using the HMQC and HMQCR Sequences,” page 184
- 5.8 “Recabling Single-Broadband Systems,” page 185
- 5.9 “Recabling Dual-Broadband Systems,” page 185
- 5.10 “Filters for Indirect Detection,” page 186
- 5.11 “Tuning the Probe in the Reverse Mode,” page 186
- 5.12 “Controlling Transmitter Power in the Reverse Mode,” page 187
- 5.13 “Indirect Detection Calibration,” page 187
- 5.14 “Typical Experimental Protocol for HMQC Experiments,” page 194
- 5.15 “Differences for 15N Indirect Detection,” page 199
- 5.16 “HSQC Experiment,” page 199

This chapter describes indirect detection experiments, also known as *heteronuclear multiple-quantum coherence* (HMQC) experiments. Indirect detection experiments show correlations between heteronuclei while detecting high-sensitivity protons. HMQC differs from the more traditional heteronuclear correlation techniques that detect the low-sensitivity heteronucleus (for example, ^{13}C or ^{15}N).

5.1 Requirements for Indirect Detection Experiments

Indirect detection experiments have three basic requirements:

- A probe with a proton channel and an X-nucleus channel.
- An rf (radio-frequency) system capable of generating pulses on ^{1}H and X and possibly decoupling X while observing ^{1}H.
- One or more pulse sequences that perform the relevant experiments.

Each of these requirements is described in more detail in the following sections.

Probes

The most commonly used probes for indirect detection experiments are the “indirect detection” probes, such as the Varian Indirect•nmr™ probe and triple-resonance probes, such as the Varian Triple•nmr™ probe. Indirect detection probes have a ^{1}H coil and an X-nucleus coil with the ^{1}H coil positioned closer to the sample for the highest possible sensitivity of the observed nucleus.

Normal “broadband” probes similarly have a ^{1}H coil and an X-nucleus coil and can be used for indirect detection. But broadband probes have significantly lower proton sensitivity (about half that of indirect detection probes) and so are not optimum for indirect detection experiments. Nevertheless, broadband probes usually provide some sensitivity improvement over direct detection heteronuclear correlation experiments. Four-nucleus and “Switchable” probes also have a ^{1}H coil and an X-nucleus coil, with the X coil closer to the sample, and can satisfy the needs for indirect detection experiments.

Probe types are not discussed further in this chapter. See the manual *Getting Started* and the probe installation manual for tuning instructions. When connecting cables to the probe, ignore words like “observe” and “decouple” and think of ^{1}H (for observe) and X (for decouple) for connections.

RF System

The rf (radio frequency) part of a spectrometer has at least four relevant aspects to indirect detection that exist in various combinations on the various systems:

- Generation of rf for the transmitter and receiver
- Amplifiers
- Filters
- Routing of rf signals

RF Generation

The first aspect is the most basic—generation of rf for the transmitter and the receiver (specifically the local oscillator or L.O. frequency). Most high-field systems, and a few specially-configured lower-field systems, are “dual-broadband” systems. Because both of the rf channels in the system are broadband, performing an indirect detection experiment involves setting the transmitter nucleus parameter to proton and the decoupler nucleus to the appropriate X-nucleus; the rf is generated correctly.

The remaining systems, including virtually all 200-, 300-, and 400-MHz systems, are “single-broadband” systems, in which the “observe” channel is broadband but the “decoupler” channel can generate only ^{1}H frequencies. These systems, therefore, must operate in a “reverse” configuration, in which the observe channel (controlled by the transmitter nucleus parameter) generates the X-nucleus decoupling frequency, while the decoupler channel (controlled by the decoupler nucleus frequency) generates the ^{1}H frequency. Insofar as generating the transmitter frequencies, this process is straightforward, but to allow the decoupler channel to be used as the reference frequency for detection (that is, to be the observe channel), the L.O. frequency must first be generated from the “decoupler” board and then properly connected to the receiver section of the spectrometer. Single-broadband systems may involve changing the cabling, which is discussed later in this manual.

Amplifiers

Amplifiers represent the second aspect of rf relevant to indirect detection. There are two configurations: systems with linear amplifiers and systems with class C amplifiers. All UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, UNITY*plus*, UNITY, broadband *GEMINI 2000*, VXR-500, and late-model VXR-S systems have linear amplifiers. Such amplifiers have computer-controlled output and can sustain power levels appropriate for X-nucleus decoupling indefinitely (*MERCURY-VX*, *MERCURY*, and *GEMINI 2000* broadband systems cannot do presaturation but can do X-nucleus decoupling).

Systems without linear amplifiers have class C amplifiers with power output that cannot be switched rapidly under computer control. Class C amplifiers preclude experiments (such as presaturation) that require power level switching during the sequence. Furthermore, on systems with class C amplifiers, the X-nucleus amplifier cannot be left on for long periods of time at the power levels appropriate for X-nucleus decoupling. If X-nucleus decoupling during acquisition is not desired, these systems pose no problem. If X-nucleus decoupling is desired, however, a limiting acquisition time of 50 to 100 ms is required unless some hardware is modified.

GARP modulation is available on UNITY*INOVA* and UNITY*plus* systems and should be used for X decoupling.

When WALTZ decoupling is used, the maximum power level for decoupling is the level that provides an rf field strength (in Hz) comparable to half the range of expected X shifts. The normal spread of protonated carbons is 150 ppm, which is 15 kHz on a 400-MHz system, and, consequently, a ^{13}C 90° pulse of 25 μs (corresponding to an rf field strength of 8 kHz) is adequate. The somewhat long proton pulses on broadband and switchable probes does not seem to present a problem because indirect detection experiments demand no more proton pulse power than DEPT or HETCOR.

Filters

Filters are the third aspect of rf that is relevant to indirect detection experiments. Filters may be needed on the transmitter, receiver, decoupler, and lock channels. Filters are part of the probe kit shipped with each indirect detection probe that Varian sells.

RF Signal Routing

The routing of rf signals is the fourth and final relevant aspect of rf. Here the major difference between systems arises from the “switchable relays” in the magnet leg. The relays are designed to allow the user to switch between ^{1}H observation and X-nucleus observation when using a normal switchable probe. Most systems are equipped with these relays, but some are not, and cable routing differs between these two configurations. An additional difference arises on systems with linear amplifiers (mostly UNITY*INOVA*, UNITY*plus*, and UNITY systems) that also have a relay intended for switching the L.O. between two sources (the “observe” and “decouple” slots). The control signal for this relay is wired on all UNITY*INOVA*, UNITY*plus*, and UNITY systems, while the rf cabling to the relay is installed on most, if not all, systems that have a factory-installed L.O. SELECT switch.

All UNITY*INOVA* and UNITY*plus* spectrometers and all single-broadband UNITY spectrometers produced after October 1990 have a configuration that allows full computer-controlled switching between direct and indirect detection. These systems have a relay in the magnet leg that accomplishes the relevant rf signal routing and computer-controlled L.O. selection, instead of the L.O. SELECT switch described above. These systems are

easily identified because most of the connectors in the magnet leg, including those attached to the relay, are N-type connectors.

MERCURY-VX, *MERCURY*, and *GEMINI 2000* broadband systems have relays to enable full computer-controlled switching between direct and indirect detection. The *GEMINI 2000* $^{1}H/^{13}C$ system does not do indirect detection.

There is a "catch" with this configuration—the filters used for indirect detection tend to degrade specifications approximately 10% in terms of longer pulse widths and lower signal/noise. The user thus faces a classic trade-off of performance (manually insert filters only when needed but achieve better specs) versus convenience (leave filters in place continuously and achieve worse specs). The convenience factor, of course, is nonexistent if the instrument does anything other than ^{13}C and ^{1}H, because one cannot leave the ^{13}C bandpass filter in place on the X line while doing ^{31}P, ^{15}N, or anything else. All standard specifications are given with the indirect detection filters *not* in place.

Pulse Sequences

There are three different data systems that users of UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, UNITY*plus*, UNITY, *GEMINI 2000*, Gemini, VXR, and XL spectrometers can have on their spectrometer systems—the Sun-based data system used with the UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, UNITY*plus*, UNITY, *GEMINI 2000*, and VXR-S (the Sun-based VXR, originally named the VXR-5000 data system), the data system used with VXR-4000 and Gemini spectrometers, and the V77-200-based data system used with XL spectrometers. This chapter covers only the Sun-based data system.

Combining the Sun-based data system with the two different rf generation types discussed previously (single- and dual-broadband) gives two fundamentally different system configurations and hence two sets of pulse sequences:

- For Sun-based dual-broadband systems, the standard system pulse sequence library contains the macros `hmqc` and `hmqcr` for setting up parameters for the HMQC and HMQCR pulse sequences, respectively. An option in `hmqc` selects another pulse sequence of interest, HMBC (Heteronuclear Multiple-Bond Coherence, the long-range sequence). Another version of HMQC, which uses decoupler 2 for the X nucleus, is also available. A compiled version of each of these sequences is in the library and ready to use; documentation on using these sequences is contained in the pulse sequence listings produced by running the macros.
- The HMQC pulse sequence can be used on single-broadband UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, *GEMINI 2000*, and UNITY*plus*. Beginning with VNMR version 5.1 (5.2F for *GEMINI 2000*), channels 1 and 2 are automatically swapped, if `tn='H1'` and `dn='X'` with channel 2 used for observe. On UNITY and VXR-S single-broadband systems, only HMQCR can be used.
- On the UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, *GEMINI 2000*, and UNITY*plus*, the sequence S2PUL pulses the decoupler channel in the "reverse" configuration if `tn='H1'` and `dn='X'`, for testing and calibration purposes. On the UNITY and VXR-S, the pulse sequence S2PULR and its corresponding macro `s2pulr` are provided for this purpose.

HMQC Pulse Sequence

The `hmqc<(isotope)>` macro sets up parameters for a HMQC (heteronuclear multiple-quantum coherence) pulse sequence. The optional `isotope` argument is the isotope number of the heteronucleus of interest, for example, `hmqc(1)` for ^{1}H (the default is ^{13}C).

Figure 39 is a diagram of this sequence. The first `2*pwx` pulse on the X heteronucleus is a composite 180 consisting of 90(`v9`) – 180(`v1`) – 90(`v9`).

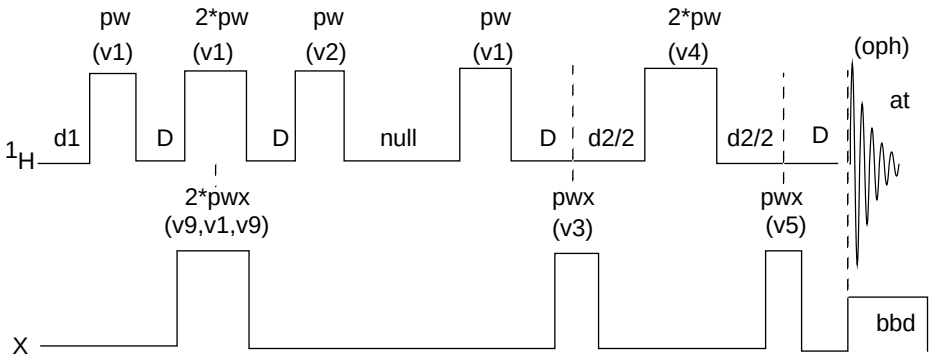


Figure 39. HMQC Pulse Sequence with `null`<>0 and `mbond='n'`

Parameters

`pw` is a 90° pulse on the observed nucleus (protons) at power equal to `tpwr`.

`pwx` is a 90° pulse on the heteronucleus at power equal to `pwxlvl`.

`dpwr` is the decoupler power level for broadband X-decouphng.

`dmf` sets the modulation frequency ($4*\gamma B_1$) at decoupler power (`dpwr`).

`dmm` is decoupler modulation mode. For UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, and UNITY*plus*, `dmm='ccg'` is recommended; otherwise, set `dmm` to `'ccw'` or `'ccp'`.

`dm='nny'` activates heteronuclear broadband decoupling (recommended) during acquisition. Note that `dm` can be set to either `'nnn'` or `'nny'`, and that the duty cycle for the decoupler should be less than 20%.

`j` is the average scalar coupling constant between the protons and the heteronucleus (usually one-bond constants). `j` is 140 for ^{13}C or 90 for ^{15}N. The time Δ, shown in Figure 39, is calculated as `1/2j`.

`null` is a WEFT-like delay used to improve the suppression of the protons connected to ^{12}C (and not to ^{13}C) that have been inverted by the preceding BIRD pulse. Try a `null` value of 0.3 for ^{13}C, 1.0 for ^{15}N, and 0 for macromolecules. To optimize, set `ss=-8` and array `null` with `nt=1` and `phase=1`. This selects the value of `null` that best minimizes the sample's signals (typically 0.2 to 0.7 seconds). If `null` is set to 0, the BIRD element is omitted from the pulse sequence.

`at` is the acquisition time (t_2 period).

`ni` is the number of t_1 increments (set up with default values for either ^{13}C or ^{15}N).

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data: if `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, –`ss` transients are applied at the start of each increment.

`nt` is a multiple of 4 (minimum) or multiple of 8 (recommended).

`phase=1,2` (2D hypercomplex data with hypercomplex-TPPI method) or `phase=3` 2D TPPI data). `phase=1,2` is suggested. For `phase=3`, remember that `hmqc` sets `sw1` to *twice* the desired value for heteronuclear experiments.

`satflg='yn'` gives presaturation during `satdly`, and `satflg='yy'` gives presaturation during `satdly` and `null` (not on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*).

`satfrq=x` is the presaturation frequency (using the transmitter), `satdly` is the length of saturation time during the relaxation period (immediately after `d1`), `satpwr` is the power level for presaturation using the transmitter (not on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*).

`hs='yn'` gives a homospoil pulse at beginning of `d1` (length=`hst`). `hs='yy'` gives a homospoil pulse at beginning of both `d1` and `null`. For *GEMINI 2000*, homospoil is not supported and `hs` must be `'nn'`.

`taumb` is a fixed delay associated with the multiple-bond HMQC experiment (`taumb=0.055` is recommended).

`mbond='n'` is a normal HMQC experiment. `mbond='y'` is a multiple-bond HMQC experiment (HMBC).

To run HMBC (`mbond='y'`): (1) set `null=0`, otherwise, only protons that are both long-range and short-range (one-bond) coupled to a given heteronucleus (^{13}C, for example) will not be suppressed, (2) set `dm='nnn'`, (3) set `taumb`, and (4) run the single-bond (HMQC) and multiple-bond (HMBC) experiments with `phase=1,2` or `phase=3`.

Phase Cycling

The phase cycling is the following:

`v1`, `v2`, `v3`, `v4`, `v5`, `v9` are phases for pulses. `oph` is the phase for receiver.

```
v1  =  x  x  y  y
v2  = -x -x -y -y
v3  =  x -x  y -y
v4  =  x  x  y  y  y  y -x -x
v5  =  x  x  y  y  x  x  y  y
v9  =  y  y -x -x
oph =  x -x  y -y
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v3`. For `phase=3`, add 90*(`ix` - 1)° to `v3`, where `ix` is the increment counter.

Technique

The usual setup is to place a ^{1}H bandpass filter between the observe port on the probe and the ^{1}H/^{19}F preamplifier, and to place a 250-MHz lowpass LC filter and either a ^{13}C bandpass or a ^{15}N bandpass filter in the decoupler line just before the probe connection.

The experiment should be performed non-spinning and with VT regulation.

HMQCR Pulse Sequence

The `hmqcr` macro sets up a HMQCR (heteronuclear multiple-quantum coherence in reverse configuration) pulse sequence. This sequence is normally used in systems with a ^{1}H only decoupler, *without automatic channel reversing*. For best results, perform the HMQCR experiment non-spinning. Figure 40 is a diagram of the sequence.

Parameters

`j` is the X-H spin coupling constant.

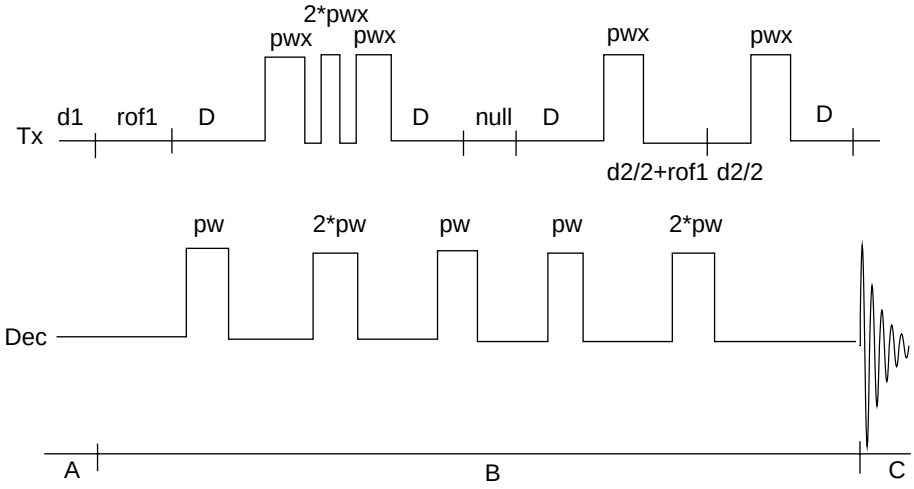


Figure 40. HMQCR Pulse Sequence

`null` is nulling time for protons not attached to X (if 0, nulling section of sequence is omitted).

`dn` and `dof` set ^{1}H (observe) frequency.

`pw` is a 90° pulse for protons.

`tn` and `tof` set X nucleus frequency.

`pwx` is a 90° pulse for X nucleus at power level `pwxlvl`.

`dm='nny'` gives software WALTZ-4 decoupling of X during acquisition; `dm='nnn'` gives coupled spectrum.

`pwxw` is a 90° pulse for X nucleus decoupling at power level `dpwr`.

`nt=8` for best results; multiple of 2 is minimum.

`ss` is the number of steady-state transients. If *ss* less than 0, then `-ss` transients are performed before *each* increment (recommended for calibration/setup experiments, not for 2D).

`phase=1,2` for hypercomplex; `phase=3` for TPPI.

5.2 The Basic HMQC Experiment

The essence of the HMQC experiment is the cancellation or elimination of the signals from protons attached to ^{12}C, leaving only signals from protons attached to ^{13}C, contributing to a ^{13}C–^{1}H chemical shift correlation spectrum. There are three basic, independent mechanisms to generate this discrimination: the spin-echo difference experiment, BIRD (Bilinear Rotation Decoupling) nulling, and presaturation.

Spin-Echo Difference Experiment

The heart of the HMQC sequence can be reduced to a heteronuclear spin-echo difference experiment that looks like Figure 41.

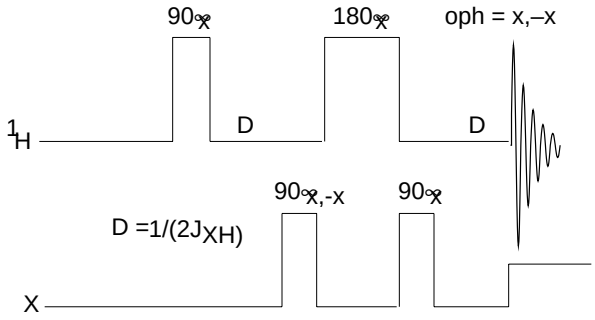


Figure 41. Heteronuclear Spin-Echo Difference Experiment

In Figure 42, *a*, *b*, and *c* represent the protons attached to carbons, where *a* are the protons attached to up-^{13}C, *b* are protons attached to ^{12}C, and *c* are protons attached to down-^{13}C. Assume that we are at the resonance frequency of the protons attached to the ^{12}Cs. In the rotating form, the following steps (shown in Figure 42) occur:

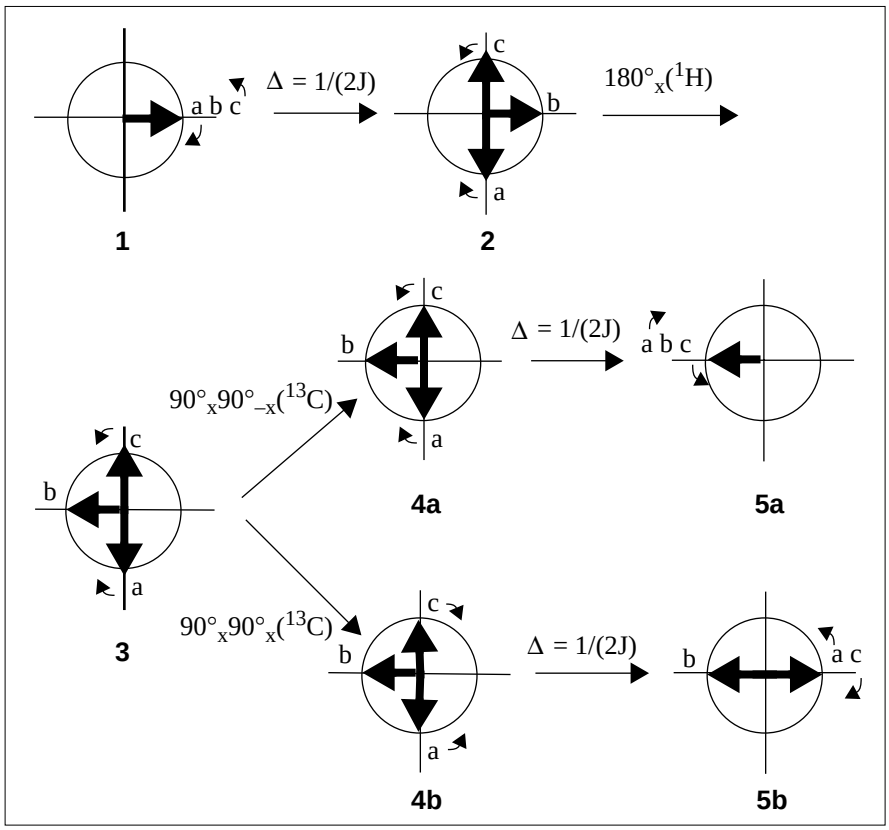


Figure 42. HMQC Pulse Sequence, Showing Movement of Attached Protons

1. The first proton 90° pulse places all protons along the *y* axis.
2. After a time Δ = 1/(2J), the *b* protons are still along the *y* axis, but the *a* protons are along the *–x* axis and the *c* protons are along the *+x* axis.

3. Next, the $180°_X$ proton pulse places the *b* protons along the –*y* axis but does not affect the *a* and *c* protons.
4. The next pulse has the following effect:
   a. The $90°_X90°_{-X}$ carbon pulse is effectively a null pulse. All rotational directions are maintained.
   b. The $90°_X90°_X$ (= $180°_X$) carbon pulse reverses the ^{13}C, which makes the *a* protons attach to the down-^{13}C and the *c* protons attach to the up-^{13}C, essentially reversing their rotational direction.
5. After another period Δ = 1/(2J), the following occurs:
   a. The *a*, *b*, and *c* protons are refocused along the –*y* axis.
   b. The *b* protons are still along the –*y* axis, and the *a* and *c* protons are refocused along the +*y* axis.

Subtracting the signal resulting from step 5b and 5a, by changing the receiver phase `oph`, results in cancellation of the *b* protons, while the signal for the *a* and *c* protons doubles.

To create a 2D experiment with information about heteronuclear chemical shifts, we introduce an evolution time t_1 that occurs between the two X-nucleus 90° pulses, as shown in Figure 43.

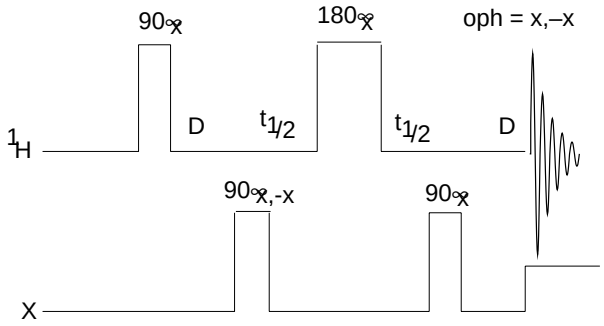


Figure 43. Evolution Time Added Between X-Nucleus Pulses

In this 2D experiment, which is now a full HMQC experiment, protons attached to ^{12}C show no different behavior and are still cancelled after two scans. For the ^{13}C nuclei, however, whether they experience a 180° pulse, a 0° pulse, or something in between, depends on the time between the two 90° pulses and their rate of precession during that time (i.e., their chemical shift). Therefore, this experiment produces a modulation of the intensity of the ^{13}C-bound protons, and the Fourier transform of that modulation yields the chemical shift of the ^{13}C bound to that proton.

In this way we detect ^{13}C chemical shifts with the intensity of protons, and simultaneously we obtain a correlation of the ^{13}C and ^{1}H chemical shifts. Appropriate variations of the experiment produce long-range coupling information.

BIRD Nulling

The second (optional) type of cancellation that can occur during an HMQC sequence is the so-called BIRD (Bilinear Rotation Decoupling) pulse nulling effect (Summers, Marzilli, and Bax, *JACS*, **1986**, *108***,** 4285). A particular sequence of the BIRD pulse, three pulses on the ^{1}H channel and one on the X channel, inverts the z-magnetization of protons bound

to ^{12}C and leaves the z-magnetization of protons bound to ^{13}C unaffected. The full sequence is illustrated in Figure 44, where $\Delta=1/2J_{XH}$.

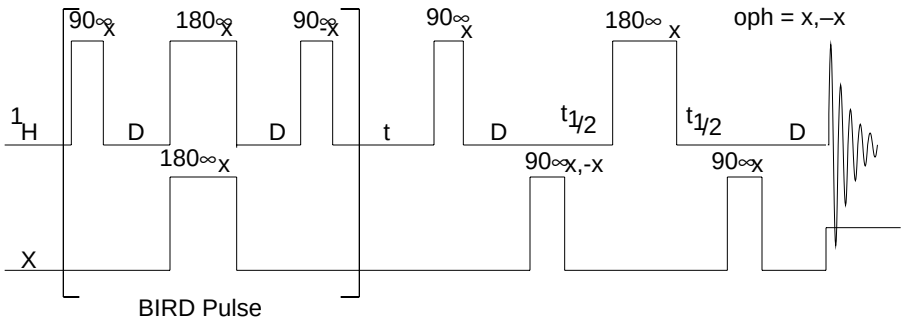


Figure 44. HMQC with BIRD Pulse Nulling Effects

After the BIRD pulse, a variable waiting period (τ in Figure 44) is inserted, allowing the ^{12}C-bound protons to relax back to equilibrium. If τ is adjusted so that the ^{12}C-bound protons are approximately at a null, then when the remainder of the pulse sequence (the normal HMQC sequence) is executed, cancellation of the ^{12}C-bound protons is enhanced (since those protons had very little magnetization at the start of the HMQC sequence). Obviously, not all protons will have the same relaxation time, so the choice of τ must be a compromise; generally, unless only one proton is involved, the additional suppression from the BIRD nulling will be a factor of two to five.

For systems that exhibit a negative NOE, such as macromolecules, cross-relaxation between the inverted protons on ^{12}C and the noninverted protons on ^{13}C will decrease the intensity of the desired proton signal. The extent of this decrease can vary between 0% and 100%. For this reason, omission of the BIRD part of the sequence is advised for macromolecules.

BIRD pulse nulling is also not possible when long-range indirect detection experiments (Heteronuclear Multiple-Bond Coherence, or HMBC) are performed. In this case, protons that have long-range couplings to ^{13}C are directly bonded to ^{12}C (99% of them, anyway) so that BIRD pulse nulling would lose all intensity in the protons of interest.

Transmitter Presaturation for High-Dynamic Range Signals

When high-dynamic range situations, such as observing signals in H_2O, are involved, HMQC phase cycling and/or BIRD pulse nulling may be insufficient to produce cancellation of the large proton signals. For this reason a third mechanism, presaturation, may be necessary. Since one channel of the instrument is set to an X-nucleus like ^{13}C or ^{15}N, this presaturation must be accomplished with the other channel; that is, the same channel that will be applying observe pulses to the protons. During one or two different periods of the sequence (during the initial delay and during the τ delay), a change in power level and possibly frequency may be appropriate in order to perform the presaturation.

In the pulse sequences discussed in this chapter, presaturation is *only* supported on UNITY*INOVA* and UNITY*plus* systems running VNMR 5.1 or later, and on dual-broadband UNITY and VXR-S systems (presaturation is not supported on fixed-frequency decoupler UNITY, VXR-S, *MERCURY-VX*, *MERCURY*, or *GEMINI 2000* systems). With appropriate pulse sequence modification, single-broadband systems with linear amplifiers could perform these experiments.

5.3 Phase-Sensitive Aspects of the Sequence

The parameter `phase`, as in other phase-sensitive 2D experiments, controls the f_1 phase detection. For 1D setup experiments or a 2D experiment without quadrature detection in f_1, use `phase=1`. For a normal 2D experiment using the States-Haberkorn-Ruben (hypercomplex) method, use `phase=1,2`. To acquire data with TPPI, use `phase=3`, and make sure `sw1` is twice the expected range.

The original HMQC experiments, including the experiments found in earlier software distributions from Varian (VNMR versions 2.2, 2.3, 3.1, and 3.2), were quite subject to axial artifacts (at f_1=0) when performed using the hypercomplex method. TPPI is preferable because the axial artifacts are shifted to the edge of the spectral region of interest (instead of falling in the center of the region of interest).

A newer technique was invented (Marion, D.; Ikura, M.; Tschudin,R.; Bax, A. *J. Magn. Reson.* **1989**, *85*, 393) involving only a change of phase cycling that shifts the axial artifacts in a hypercomplex experiment to the edge of the spectrum, giving the hypercomplex version the benefit of TPPI with none of the disadvantages. We have termed this technique, which is applicable to all 2D experiments, the name FAD, for “F1 Axial Displacement.” It is also referred to as “States-TPPI.” The `hmqc` and `hmqcr` macros in VNMR version 4.1 and later include FAD. Once implemented, use of the technique is totally transparent—just perform a standard hypercomplex experiment with `phase=1,2`.

5.4 Cancellation Efficiency

Because indirect detection experiments involve cancellation of non-^{13}C-bound protons that are two orders of magnitude more intense (assuming unlabeled compounds), cancellation efficiency is critical. Cancellation efficiency, in turn, depends on the fundamental stability of the system rf and the reproducibility of anything else that can affect the signal. While stability is fixed by the instrumentation, you can control a number of operating conditions that can influence the quality of any cancellation experiment (NOE difference experiments are another good example). Some of these conditions are discussed here, roughly in order of importance:

- Run experiments non-spinning. This is a must.
- Use the highest lock power at which the lock is stable (be sure to shim with a non-saturating level, however) and keep the lock gain as low as possible, sufficient only to be sure that you don’t lose lock during the experiment.
- Use a ^{2}H band-pass filter in the lock line. Interference between X-nucleus decoupling or even X-nucleus pulses can affect the lock and cause field instabilities, limiting the ability to perform cancellation experiments (if you don’t have such a filter and want to prove this to yourself, try a short-term experiment in the unlocked mode).
- Use VT regulation, even at room temperature. Large changes in temperature of the environment can affect the VT gas stream. The frequency of peaks in the spectrum and of the lock resonance (which affects all peaks) is temperature-sensitive to some degree. Shimming may also change if the probe temperature varies, which can affect the lineshape.
- Be sure the system is in thermal equilibrium. If you are performing experiments with X-nucleus decoupling, you are applying large amounts of power to the system, which is almost certain to change the temperature of the probe, the sample, or both, even when you are performing VT regulation. The best way to ensure thermal equilibrium is to set up a “dummy” experiment with *identical* conditions (in terms of duty cycle) to your actual experiment, but which runs for perhaps several minutes (easily

accomplished by setting *ni* to a small number). Now, if you queue your real experiment to follow the dummy one, the sample and probe are properly equilibrated.

- Be sure the system is in an NMR steady-state by using steady-state pulses.
- Use a large value of `nt`. Cancellation improves with larger `nt`, so the relevant cancellation is that which occurs at `nt` comparable to what you will be using in an indirect detection experiment (16 to 1024). Do not expect perfect results with `nt=2`.
- Minimize floor vibration. Where this cannot be fixed by spectrometer placement, an antivibration system should be installed.
- Use a moderate flow of body air through the probe to eliminate “rattling” from turbulent flow.
- Use lengthened pulses (attenuated rf) if you have a rise time or phase glitch problem.

Before beginning an HMQC experiment, you should assess the quality of your reproducibility by performing some simple difference experiments. The standard S2PUL pulse sequence is a good one to use for this purpose. The first pulse of S2PUL, controlled by `p1`, is held at a constant phase, while the receiver varies in phase. Thus, after four scans with `p1` set to the 90° value, `pw=0`, no signal should be seen. This can be compared to four scans with `pw` set to the 90° value, `p1=0`, which produces a full signal. Taking the ratio of these two spectra gives a concrete measurement of your cancellation efficiency, while repeating the null spectrum a number of times gives a measure of the reproducibility of the cancellation. Use this test to assess the value of the various steps and modifications described above, or of other differences (for example, the relative cancellation efficiency of experiments with and without X-nucleus decoupling).

Because rf stability is an issue and rf generation on single-broadband systems differs between observe and decoupler channels, on systems prior to the UNITY*plus* you should repeat the same experiment using S2PULR to pulse the decoupler channel (on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* running VNMR 5.2F or later, and on UNITY*INOVA* and UNITY*plus* running VNMR 5.1 or later, use S2PUL with `tn='H1'` and `dn=X` instead of both `tn` and `dn` set to `'H1'`). While the overall cancellation ratio depends on all factors (vibration, lock channel, etc.), the ratio of the cancellation achieved gives you a measure of the relative performance of your decoupler channel in comparison to the observe channel.

5.5 Pros and Cons of Decoupling

It may seem that decoupling of X during acquisition is always better. The advantages are clear—the spectrum is less crowded, with only half as many peaks, and each peak has twice the sensitivity. This is, however, a simplistic view.

The disadvantage of X-nucleus decoupling stems from the need to use large (up to 8 kHz) decoupling fields. This high power can cause significant heating, particularly in lossy samples. As a consequence of sample heating, experiments with X-nucleus decoupling are generally limited to relatively short acquisition times, which in turn may produce less resolution in f_2 as well as less sensitivity for molecules with long T_2. Furthermore, the heating that does occur frequently produces worse cancellation efficiency. And finally, to prevent the buildup of heat in the sample, the duty cycle of the experiment may need to be limited to 10 to 20%, again possibly reducing sensitivity. For all these reasons, experiments performed without X-nucleus decoupling are perfectly reasonable, and may well be preferable.

If X-nucleus decoupling is desired, it is important to avoid sample heating. This form of sample heating can be non-uniform within the sample and can cause microconvection,

producing poor cancellation. Keep the acquisition time short and the overall duty cycle less than 20%. To lower the decoupler power to tolerable levels, it may be necessary to add a fixed 6-dB attenuator to the X-nucleus channel on systems in which that power is not under computer control; this can have the unavoidable consequence of lengthening the pulse widths of the X-nucleus pulses.

Dual-broadband and *GEMINI 2000* broadband systems have no problem performing modulated decoupling, because the decoupling is being performed by the normal spectrometer decoupling channel. On single-channel broadband UNITY and VXR-S systems, however, the decoupling is being performed by the normal observe channel, and the standard modulation (WALTZ, for example) is not present.

In the sequences described here, broadband decoupling is achieved by using the acquisition computer to provide WALTZ-4 modulation of the X-nucleus channel through explicit software control. This requires hardware looping capability and explicit acquisition, which UNITY and VXR‘-S systems support. It also imposes some limitation on spectral widths and pulse widths, since the WALTZ-4 sequence (whose length is `6*pw90`) must fit in between successive data point samplings (which occur at time intervals of `1/sw`).

GARP modulation is available on UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, and UNITY*plus* systems and should be used for X decoupling.

5.6 Specifications Testing

Before performing any calibrations or specifications testing, connect the cables to the system as if it were a standard broadband probe, and tune both ^{1}H and X-nucleus channels of the probe just as you would a broadband probe, using tuning procedures described in the indirect detection probe section of the probe installation manual. Now using the standard samples and standard tests (see the acceptance tests procedures manual for the system), measure the pulse width, lineshape, linewidth, spinning sidebands, and ^{1}H signal/noise.

Note: All specifications on Varian indirect detection probes are guaranteed in the “normal” (i.e., direct observation) configuration. Changes in cabling, filters, and signal routing for indirect detection experiments may affect performance.

Before proceeding for the first time to indirect detection experiments on single-channel broadband systems, one basic test is important: verify that the local oscillator (L.O.) signal from the decoupler board is both enabled and of sufficient amplitude and quality to produce an acceptable NMR signal. The steps below show how to do so:

1. Measure the S/N of the system using any sample (the standard S/N test sample is useful but certainly not required).
2. Use `s2pul` with `tn='H1'` and `dn=X` (`X` can be `'C13'`) to switch to the “reverse” mode, changing the cabling if necessary (described later in this chapter) for UNITY*INOVA* and UNITY*plus* running VNMR 5.1 or later, and on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* running VNMR 5.2F or later. Use S2PULR on single broadband UNITY and VXR-S.
3. Recalibrate the 90° pulse width, and check the setting of the `gain` parameter to ensure that you are filling the ADC with the signal (using `ddff(1)` to digitally examine the FID).
4. Measure the S/N again in the reverse configuration. The measured value should be *approximately* the same as in the normal mode. Values in the range of ±15% from the “normal” value seem typical. If the S/N drops more than 15%, the L.O. signal on the decoupler board probably needs to be adjusted.

5.7 Using the HMQC and HMQCR Sequences

Figure 45 shows again a diagram of the basic HMQC sequence. The parameters that correspond to this diagram depend on which sequence is used—HMQC or HMQCR. Table 32 lists the associated parameters.

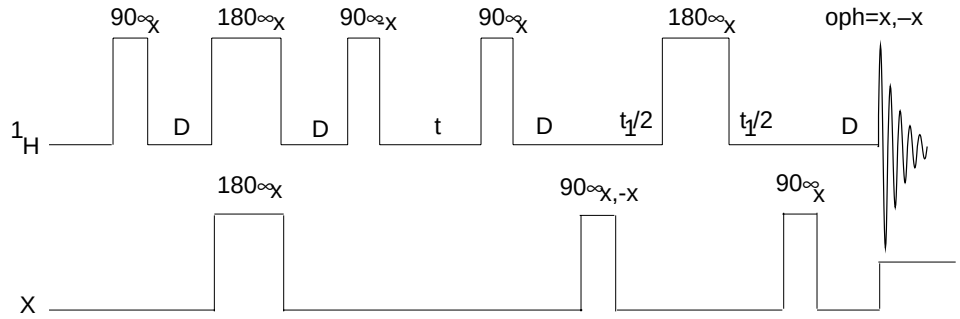


Figure 45. Basic HMQC Pulse Sequence

Table 32. Parameters for HMQC and HMQCR Pulse Sequences

Parameter	*HMQC*	*HMQCR*
^{1}H 90° pulse	`pw`	`pw`
^{1}H 180° pulse	derived from `pw`	derived from `pw`
^{1}H amplifier power (if appropriate)	`tpwr`	`dpwr`
^{1}H frequency	`tn, tof`	`dn, dof`
^{1}H spectral width	`sw`	`sw`
X 90° pulse	`pwx`	`pwx`
X 90° pulse for WALTZ decoupling	`1/(4¥dmf)`	`pwxw`
X 180° pulse	derived from `pwx`	derived from `pwx`
X amplifier power for pulses (if appropriate)	`pwxlvl`	`pwxlvl`
X amp power for decoupling (if appropriate)	`dpwr`	`tpwr`
X frequency	`dn, dof`	`tn, tof`
X spectral width	`sw1`	`sw1`
Δ delay	`1/(2j) [if j=0, D=0]`	`1/(2j)`
τ delay for BIRD nulling (if `null=0`, entire BIRD sequence is skipped)	`null`	`null`
Coupled experiment	`dm='nnn'`	`dm='nnn'`
X decoupling during acquisition	`dm='nny'`	`dm='nny'`
Setup experiments	`phase=1`	`phase=1`
Hypercomplex experiment	`phase=1,2`	`phase=1,2`
TPPI	`phase=3`	`phase=3`
Minimum `nt` possible	`2`	`2`
Presaturation and/or multiple-bond correlation	see text	not applicable
Axis parameter for proper ppm on both axes	`pd`	`dp`

5.8 Recabling Single-Broadband Systems

On systems with computer-controlled switching between normal and reverse modes (described above), the spectrometer switches automatically to reverse mode when you use either HMQCR or S2PULR sequences. If you have such a system, ignore any further recabling instructions in this chapter.

MERCURY-VX, *MERCURY*, and *GEMINI 2000* have automatic switching. If you have a *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* broadband system, skip to "Filters for Indirect Detection," page 186.

To manually switch single-broadband systems to a reverse configuration, perform the following steps:

1. Toggle the **L.O. SELECT** switch that enables the local oscillator (L.O.) gate on the decoupler channel of the instrument. This switch is installed as follows:
   - As part of the Indirect Detection Modification Kit 00-990841-00 (see *Magnetic Moments* Vol. IV, No. 2, p. 16)
   - By service personnel using *Varian Service Bulletin MP880002*
   - Customer-installed using *Magnetic Moments* Vol. III, No. 4, p. 7
   - Factory-installed
2. Unplug the L.O. cable from the transmitter board (J2X5 on UNITY*INOVA* or UNITY*plus*, J3402 on other systems) and plug it instead into the L.O. connector of the decoupler board (J2X5 on UNITY*INOVA* or UNITY*plus*, J3302 otherwise).
3. Reconnecting the cables carrying the transmitter and decoupler signals depends on the system configuration. You may need to try more than one variation to find the correct method for your system; this can be done by either using the HMQCR pulse sequence itself or by using an oscilloscope:
   - Spectrometers shipped after October, 1990, with "N-type" connectors, should require no recabling.
   - The following method should work on earlier UNITY spectrometers: Disconnect the decoupler cable from the Bird wattmeter inside the magnet leg, and connect it directly to the X-decouple input to the probe (via the appropriate filter).
   - The following method has been found applicable on some systems: Reverse the observe and decoupler inputs to the probe; that is, take the cables (and associated filters) normally connected to the 1H/OBS/DEC BNC jack and the NORMAL jack on the inside of the magnet leg and reverse them.

5.9 Recabling Dual-Broadband Systems

Because dual-broadband systems are operated in the "normal" mode, there is little recabling necessary (with the exception of filter placement; see the next section). Both lock and ^{1}H observe signals follow their normal pathway.

The X channel, however, does have to be recabled. Under conventional (direct observation of the X-nucleus) circumstances, the X channel of the probe will be connected to the NORMAL jack on the inside of the magnet leg; for indirect detection, the X channel of the probe must be connected to the (now mislabeled) 1H/OBS/DEC jack. (The above information applies to 500-MHz and 600-MHz system only; dual-broadband systems at other frequencies require different cabling).

5.10 Filters for Indirect Detection

A number of filters are necessary for optimum performance of indirect detection experiments, regardless of whether a single- or dual-broadband system is used.

In the lock channel line, install a ^{2}H band-pass filter. When the filter is added, expect the lock phase to change. This filter can be left in the system at all times; it will, however, cause a small (about 3 dB) loss in lock sensitivity.

Route the cable attached to the probe connector marked {x} decouple through an X-nucleus band-pass filter. Route the cable attached to the ^{1}H OBSERVE spigot through a ^{1}H high-pass filter; route it through a ^{2}H band reject filter as well (not supplied) if 2 kHz lock birds are seen (5 kHz on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*).

Table 33 lists part numbers for the filters supplied with UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, UNITY*plus*, *GEMINI 2000*, and UNITY indirect detection probes. If you are performing indirect detection experiments on other probes (Varian broadband or switchable probes, or probes purchased from other vendors), you may need to purchase these filters separately.

Table 33. System Bandpass Filters for Indirect Detection Probes.

Filter	*300-MHz System*	*400-MHz System*	*500-MHz System*	*600-MHz System*	*750-MHz System*
^{15}N	BE30.4-7.6-9BB	BE40-10-9BB	BE53-15-8BB	BE61-10-8BB	BE77-15-4BB
^{2}H	BE46-4.5-6BB	BE61-10-8BB	BE77-3.8-8BB	BE92-9-6BB	BE115-11-6BB
^{13}C	BE75-15-8BB	BE109-22-8BB	BE135-35-8BB	BE151-40-8BB	BE188-20-7BB
^{31}P	BE135-35-8BB	BE151-40-8BB	BE175-60-8BB	BE240-100-8BB	BE301-46-8BB

5.11 Tuning the Probe in the Reverse Mode

The probe can easily be tuned once the recabling described above is performed. On *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, use a separate experiment with the standard parameters to tune the probe (see the *NMR Probes Installation* manual for further information).

Tune the ^{1}H Channel

1. Make sure the spectrometer is transmitting the proton frequency by entering **`tn='H1' su`**.
2. Connect the cable that comes from the ^{1}H channel of the probe to the TUNE connector on the preamplifier or magnet leg.
3. Tune as described in the probe installation manual. After tuning, put the cables back the way they were.

Tune the X Channel

1. Enter the nuclei for the X channel (e.g., to use ^{13}C, enter `tn='13C' su`).
2. Connect the cable that comes from the X channel of the probe to the TUNE connector on the preamplifier or magnet leg.

3. Reconnect the cables containing the transmitter and decoupler signals as described in step 3 on page 185.
4. Tune as described in probe installation manual. After tuning, put the cables back the way they were.

5.12 Controlling Transmitter Power in the Reverse Mode

On a Sun-based system, you will generally find that the parameter `dpwr` is limited to a value of 49 to prevent excessive decoupling power. When `dpwr` is used to control the ^{1}H transmitter power, as it is in the S2PULR and HMQCR sequences, this will be insufficient power. To remove this limitation, you can use the `config` program to reset the Upper Limit of the coarse attenuator for the decoupler.

5.13 Indirect Detection Calibration

On both single- and dual-broadband systems, calibrations are not the same after cabled for indirect detection as in "normal" mode, and so it is necessary to calibrate the system after recabling. The first system calibration should be done with the standard sample (Part No. 00-968120-96), which contains 1% $^{13}CH_3I$ (as well as a number of other species). This sample also enables running a quick HMQC spectrum to verify overall operation of the system.

Note: Throughout the following instructions, refer to Table 32 to see which parameters control the features in your configuration.

1. The first time you perform this experiment, cable the system for normal ^{13}C observe, and obtain a normal ^{13}C spectrum in the usual way. The ^{13}C signal from $^{13}CH_3I$ is extremely far upfield (22.3 ppm), so depending on your standard parameters you will probably need to either increase your spectral width or move your transmitter offset upfield (to small or more negative numbers) to prevent foldover of the signal. A typical result is illustrated in Figure 46 in which the peak at 22 ppm is the $^{13}CH_3I$ signal. Note that the decoupler should be on. Increase scans for low field systems (for example, 16 for 300 MHz).

   Once you have identified the signal 22 ppm (it should be easily visible with a single pulse—this is a 50% enriched sample!), position the cursor near the line and type `nl movetof`. The software will change the transmitter offset `tof` so that it is on-resonance for this ^{13}C resonance. Make a record of this value of `tof` for future use. Now, if needed, re-cable the system to the indirect detection configuration.

2. Obtain a normal ^{1}H spectrum. On the UNITY*INOVA*, *MERCURY-VX*, *MERCURY*, *GEMINI 2000*, UNITY*plus*, and dual-broadband UNITY and VXR-S systems, use S2PUL with `tpwr` set to the desired value (it will also be active in HMQC), `tn` set to `'H1'`, and `dn` to the appropriate X nucleus. On single-broadband UNITY and VXR-S systems, use S2PULR to pulse the decoupler, positioning the spectrum with `dn` and `dof` the same as in the indirect detection experiment (and also set `tn` to the proper X-nucleus at this time). S2PULR pulses the decoupler at a power level controlled by the parameter `dpwr`.

   If you observe a proton spectrum, you have confirmed that you have properly re-cabled the ^{1}H channel. Using the `movesw` command, narrow the spectral width to the region containing the full proton spectrum, as seen below (note that if you are operating in the reverse mode with S2PULR, the commands `movesw` and

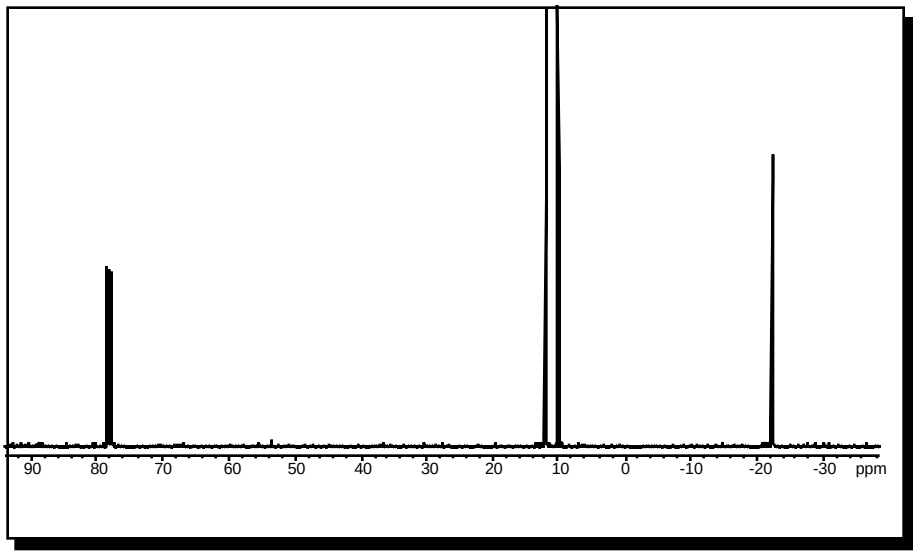


Figure 46. Normal ^{13}C Spectrum of $^{13}CH_3I$

`movetof` are not appropriate, since they change the value of `tof` rather than `dof`. Either recalculate the proper values "by hand," or use a series of commands like `r1=tof tof=dof movesw dof=tof tof=r1`).

The large three-line pattern (approximately 1:1:1) in the spectrum, illustrated in Figure 47, (centered at 2.2 ppm) represents unenriched CH_3I (the central line) and

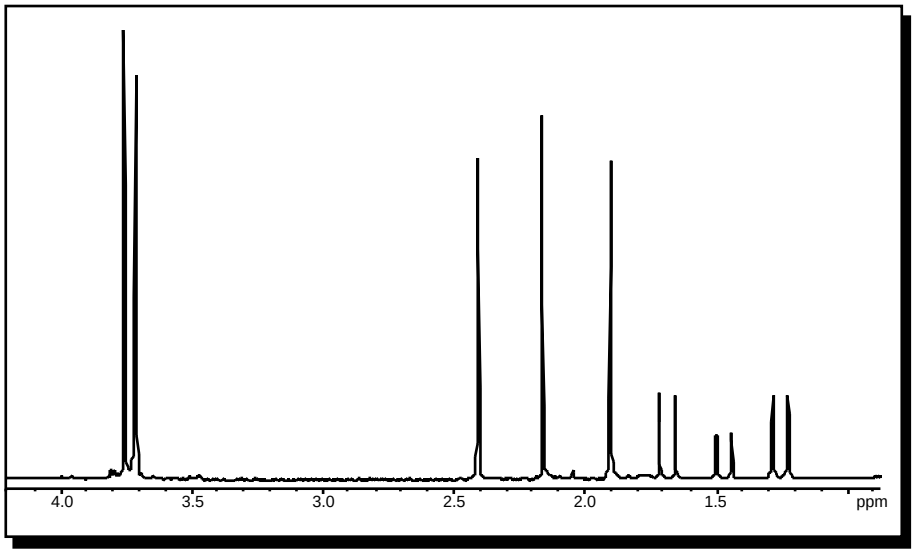


Figure 47. Normal ^{1}H Spectrum of $^{13}CH_3I$

the enriched $^{13}CH_3I$ (the outer two lines). The peaks centered at 1.5 ppm in the spectrum are a ^{13}C enriched impurity peaks, and the peaks near 3.7 ppm are trimethylphosphite, which is also present in the sample (for ^{31}P indirect detection experiments). Now calibrate the proton pulse width `pw` in the usual way.

3. Enter **`hmqc`** or **`hmqcr`** (as appropriate) to set up the HMQC experiment.

Before you proceed any further, make sure the spinner is off by setting **spin=0** or by using the acqi window. Listen for the spinner air to turn off and check in the acquisition status window that the spinner is off and actually shows 0 Hz.

4. Verify that the proton amplifier power (if appropriate) and pw are what you established in step 2. If you have a linear amplifier on your X channel, set its pulse power level with **pwxlvl**. Set the position of the ^{13}C channel to the on-resonance position measured in step 1. Enter **phase=1 ni=1 nt=1 dm='nnn' null=0 ss=0 j=151 spin=0 ai pwx=0,15 wexp='wft dssh' au**.

   Two spectra are collected. In the first, no X-nucleus pulses are used (pwx=0), so a normal spectrum should appear (inverted); in the second, X-nucleus pulses are used, and if the value of pwx is correct, the ^{13}C satellites of the $^{13}CH_3I$ should appear right side up, as seen in Figure 48. If the two spectra are identical, you have either

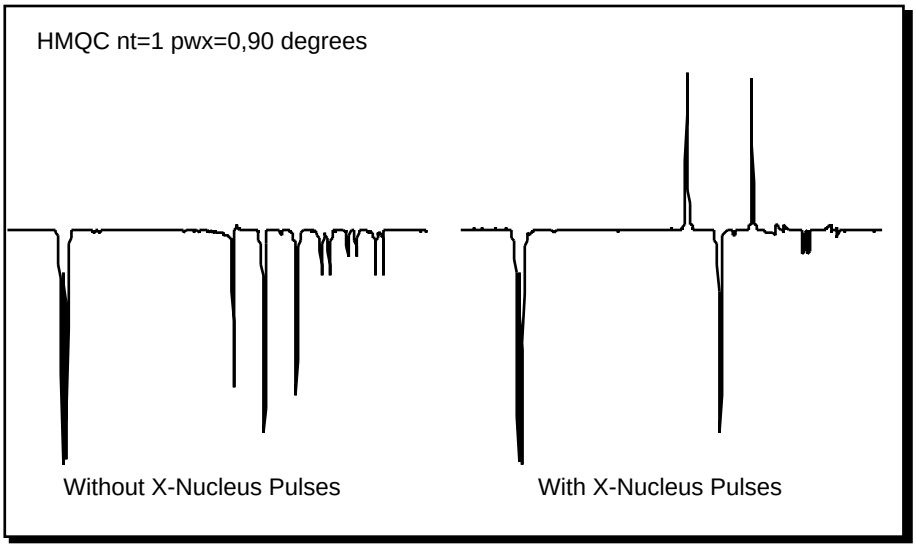


Figure 48. HMQC Without and With X-Nucleus Pulses

entered a value for pwx that is exactly 360° (unlikely but you can easily find out), you have not cabled the X channel correctly, or dof might be set wrong.

Note two occurrences in this experiment:

- Because the pulse sequence involves spin echoes whose timing may not be perfectly adjusted, it may not be possible to perfectly phase the first spectrum; the spectrum on the left in Figure 48 is typical. If the spectrum phases poorly, recheck the proton 90° pulse width.
- Because the pulse sequence is very much optimized for a particular coupling constant, the ^{13}C satellites of the upfield impurity, which have a coupling of 128 Hz, exhibit a strange phase in the spectrum obtained with a 90° *pwx* pulse on the right. This is a consequence of the sequence and you should not attempt to phase this spectrum "properly;" since you will be unable to do so.

5. Assuming you see a difference between the two spectra in step 4, perform an array of values for pwx and select the one for which the ^{13}C satellite signals are maximized (make sure to set d1 sufficiently long for this experiment, say, 10 seconds). In the spectra shown in Figure 49, the series on the left represents a broad array of pwx values from 0 to 52 µs in steps of 4 µs. Such a series can be used to get the "big picture" when you have no idea of the proper value of pwx.

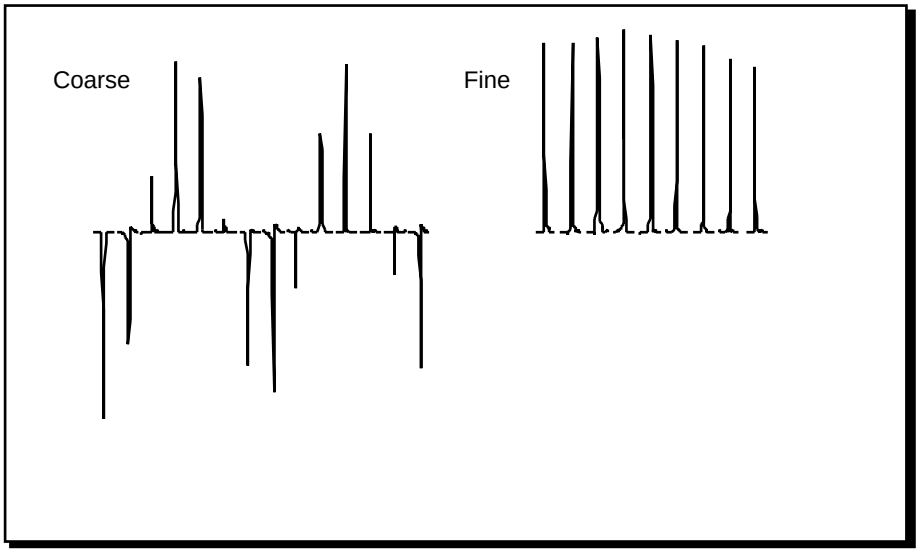


Figure 49. Calibration of pwx, Coarse and Fine

Note that unlike a standard pulse calibration, the "null points" in this calibration are *not* the points of interest. The signal starts out inverted at 0°, becomes maximally upright at 90°, inverts at 180°, etc. In the series on the right, *pwx* has been varied from 12 to 16 µs in steps of 0.5 µs to more properly select the 90° value. Note also that this calibration will be *severely* impacted if the X-nucleus transmitter is not placed on-resonance during the experiment, even though the nucleus you are observing is ^{1}H. Note that the view is set to `ai` (absolute intensity) so that peak heights can be compared.

The calibration of `pwx` can also be done with the **`pwxcal`** macro and sequence (not supplied with the *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*). Refer to the manual *Getting Started* for the procedure.

6. For systems without linear amplifiers, skip to step 7.

   For systems with linear amplifiers, the following is optional: Increase the power of the amplifier (using **`pwxlvl`**) by 1 or 2 units and repeat step 5. `pwx` should decrease as you increase the X-nucleus pulse power. When `pwx` no longer decreases, the probe is arcing; decrease the power by 3 units to be safe and do not go above this value. Proceed to step 7.

7. Check cancellation. Set **`nt=4 d1=10 ss=1`** and array **`pwx`** with the 0° and 90° pulse widths. The two spectra that result should show no signal in the first case and ^{13}C satellites only in the second case. If the cancellation is worse when the X-nucleus pulses are present, as in Figure 50, this is an almost certain indication of a lock interference problem. The spectrum in Figure 50 was performed without a ^{2}H band-pass filter on the lock channel. Compare it to the spectrum in Figure 51, a repeat of the same experiment with a ^{2}H band-pass filter.

   When you repeat this experiment on a "real" (i.e., natural abundance) sample, the difference between these two spectra will give you confidence that what you are observing are indeed ^{13}C satellites and not residual uncanceled signals.

8. Choose either step a or b—on systems other than single-broadband UNITY and VXR-S, perform step a and skip step b below. On single-broadband UNITY and VXR-S systems, skip step a and perform step b.

   After this step, the carbon parameters need to be calibrated.

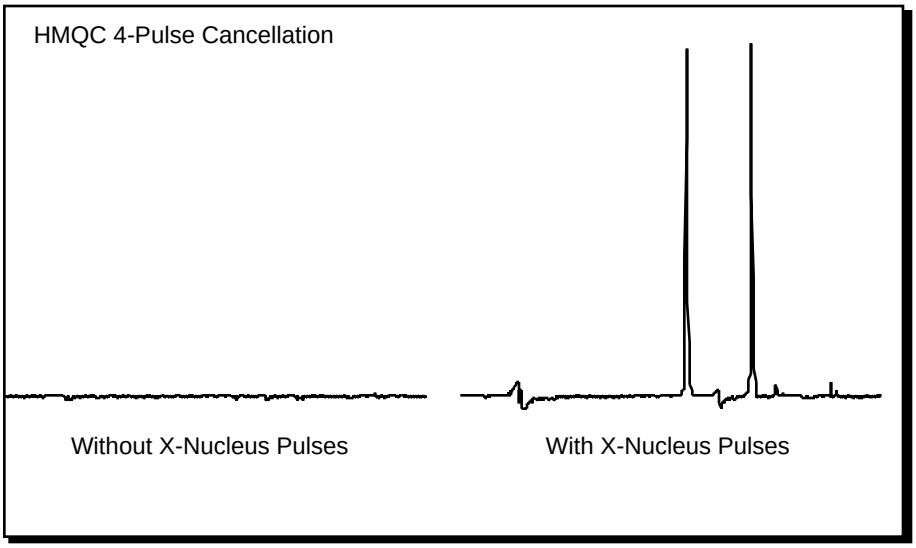


Figure 50. HMQC 4-Pulse Cancellation without ^{2}H Bandpass Filter

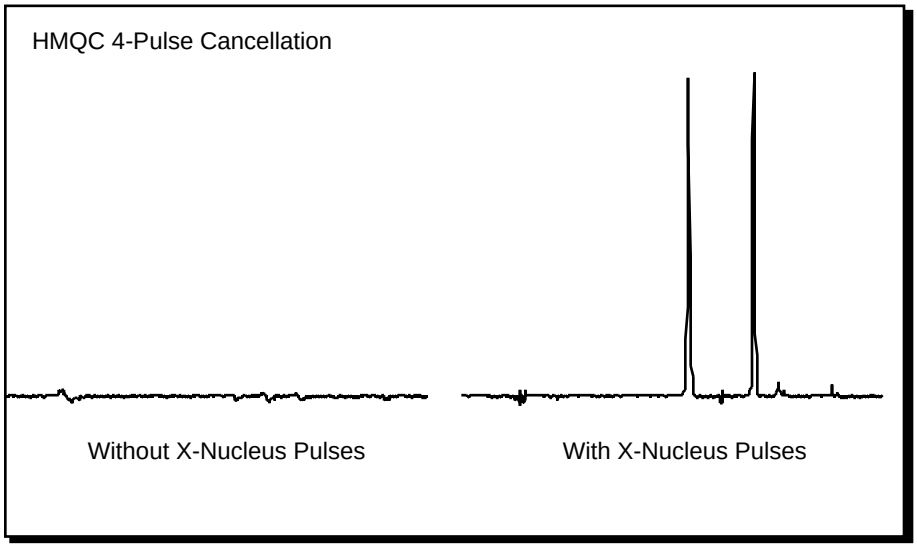


Figure 51. HMQC 4-Pulse Cancellation with ^{2}H Bandpass Filter

a. *Systems other than single-broadband UNITY and VXR-S* – Modify the parameters to perform two single-frequency, off-resonance decoupled experiments in order to calibrate γB_2. Set **`axis='h' dpwr=43 dm='nny' dmm='c' at=0.256 lb=2`**. Enter an array of **`dof`** values that are ±4000 Hz from the on-resonance position. `h2cal` returns γH_2=xxxhz, pw90 at that power, and coelesc frequency. Set dmf=1/90 or 4*γH_2.

   Obtain two spectra and measure the residual coupling between the two lines. To do this, enter **`ds(1)`**, place two cursors on the two outer lines, and enter **`r1=delta`**. This will save the first splitting in the variable `r1`. Now enter **`ds(2)`**, place two cursors on the two outer lines, and enter **`r2=delta`**. Now enter **`h2cal(r1,r2,151)`** (151 is the full coupling constant), and the

computer will display the value of γB_2 (to obtain a printed copy, enter **printon h2cal(r1,r2,151) printoff**).

Increase or decrease **dpwr** by 1 or 2 units and repeat step 2. The highest possible value of dpwr that you will be able to use for WALTZ or GARP modulated decoupling may be limited by the maximum decoupler modulation frequency, dmf. For ^{13}C indirect detection, this will be a reasonable power level if you wish to decouple the full ^{13}C spectrum.

If you wish to decouple only part of the ^{13}C spectrum, you should decrease γB_2 accordingly; the lower the amount of decoupler power used, the less sample heating and convection that will occur, and the better will be your cancellation in indirect detection experiments. The greater the decoupling power used, the more critical it is to establish a temperature steady-state in the experiment and to keep the duty cycle low (see discussion above in the section “Cancellation Efficiency”).

b. *Single-broadband UNITY and VXR-S systems* – Now that you are convinced you are seeing ^{13}C satellites, check your decoupling. Set **pwx** to the 90° value, **pwxw** to the same value and **tpwr=pwxlvl dm='nnn','nny' at=0.256 lb=2 au**. These two spectra should now both contain ^{13}C satellite signals, in the first case coupled, and in the second case ^{13}C-decoupled, as seen in Figure 52.

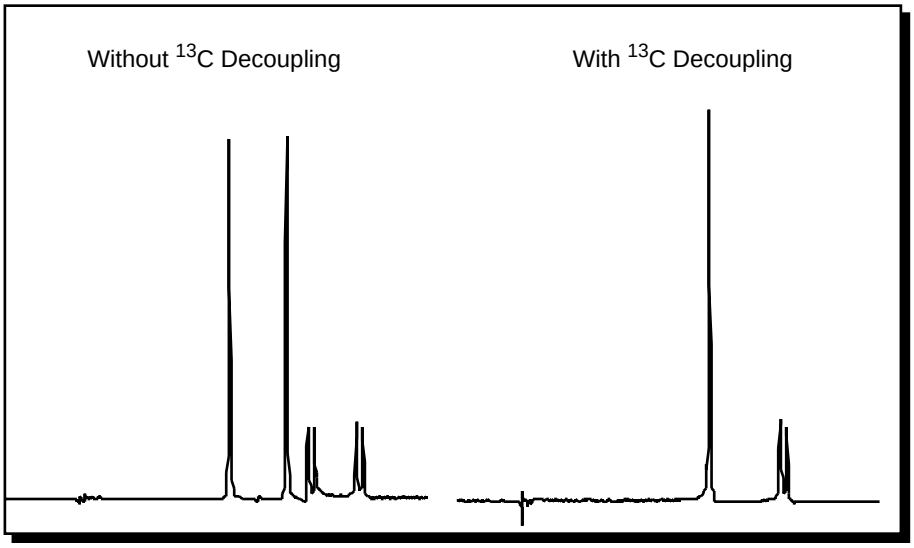


Figure 52. HMQC Without and With ^{13}C Decoupling

Note that these two spectra may not have precisely the same phase, due to the nature of the pulse sequence; this is of no consequence. Further note that the peaks in the decoupled spectrum, even after optimizing the decoupling, will generally only be approximately the same size or slightly larger than the coupled spectrum, although you might have expected a peak twice as large. ^{13}C decoupling requires the ability to spread the decoupling power over a very wide range, and even with modern decoupling techniques such as WALTZ, this is typically accompanied by a line broadening in the decoupled spectrum compared to the coupled spectrum.

Once you have verified decoupling is functional by comparing these two spectra, set **dm='nny'** and array **pwxw** to find the value that gives optimum

decoupling. This value should be close to `pwx` but may be a few microseconds longer.

We started with the X-nucleus power the same for pulses (`pwxlvl`) and decoupler (`tpwr`). It may be desirable to use less power for decoupling (see "Cancellation Efficiency," page 181). If you wish to do so, you should decrease **`tpwr`**. For every 3 units (3 dB) `tpwr` is decreased, `pwxw` by should be increased by about a factor of $\sqrt{2}$ (1.414), or by a factor of 2 for a 6 dB decrease in `tpwr`. A constraint is placed on the length of `pwxw` by the fact that `6*pwxw` must be less than `1/sw`.

9. You have now determined proper values for a number of parameters. A good idea is to save the parameters in one of the following ways:
   - Enter the parameters into the appropriate probe calibration file.
   - Join another experiment and recall the standard parameters by entering **`rtp('/vnmr/parlib/hmqc13')`** or **`rtp('/vnmr/parlib/hmqcr')`**. Enter the correct values for the various parameters and save the parameter set by using `svp` to save the parameters in a file of the same name. Now these parameters will be reestablished when you use macros to set up future experiments.

10. Perform a simple 2D experiment on the enriched sample. Starting with the experiment in which you have been performing all the calibrations, set **`ni=128 nt=4 phase=1,2`**. Move the ^{13}C transmitter position 17 ppm downfield (to high frequency) from the on-resonance position for the $^{13}CH_3I$ by entering **`dof=dof+17d`** (for `hmqc`) or **`tof=tof+17p`** (for `hmqcr`). Set the ^{13}C spectral width `sw1` to 50 ppm by entering **`sw1=50d`** (for `hmqc`) or **`sw1=50p`** (for `hmqcr`). To acquire a coupled 2D spectrum, set **`dm='nnn'`**; to obtain a decoupled spectrum, set **`dm='nny'`**.

    Now acquire the data by entering **`go`**. Following acquisition, set **`fn=2*np fn1=512 gf=.5*at`** and **`gf1=.5*ni/sw1`** and process with **`wft2da`**; the result should look something like Figure 53.

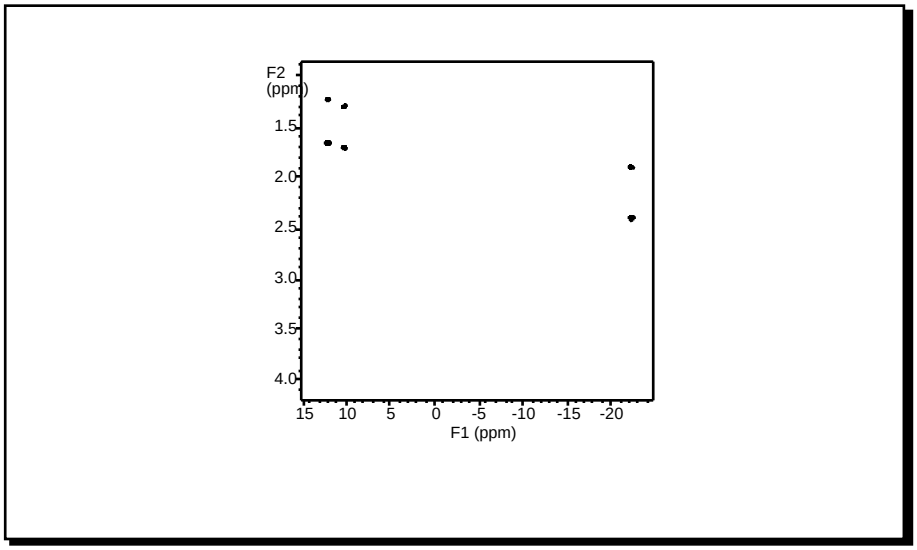


Figure 53. Coupled HMQC Spectrum of $^{13}CH_3I$

11. When processing the data, one of the trickiest aspects of this experiment is phasing in the f_1 direction (phasing in f_2 is accomplished simply by performing a 1D transform on the first increment with `wft(1)` and phasing the spectrum, paying attention only to the ^{13}C satellite peaks). In F1, the combination of the usually large spectral width and the pulse in the center of the evolution time produces large negative values for `lp1`.

    Reasonably good starting points for the F1 phase can be calculated according to the following formulas:

    `lp1= -sw1 × 360° × (2*rof1+2*pw+4*pwx/p` (for `hmqcr`)
    `lp1= -sw1 × 360° × (4*pwx/p)` (for `hmqc`)
    `rp1= -lp1/2`

5.14 Typical Experimental Protocol for HMQC Experiments

To run HMQC experiments on a “normal” sample, you use a procedure similar to that described above for the standard (enriched) sample. The following instructions mostly emphasize the small differences in operation. Where the instructions are cryptic, refer back to the instructions in the previous section for detail.

A good “normal” sample to use for your first natural abundance sample is the Varian ^1H App Test sample (Part No. 00-968120-93), which is 1% 3-heptanone in $CDCl_3$.

Throughout the following instructions, refer to Table 32 to understand which parameters control the features in your configuration.

1. Insert the sample and, after shimming, leave the spinner off. If you are going to run the experiment at a controlled temperature, enter **`temp=x su`** to start the temperature regulation process.

2. Set up to obtain a normal carbon spectrum and narrow the spectral width to the appropriate region. In some cases, the ^{13}C spectrum will be too weak to observe in a reasonable amount of time. To set the parameters controlling the ^{13}C frequency and spectral width if this is the case, you can take two approaches. First, if you have done similar experiments in the past on similar samples, just use the same parameters. Alternatively, if you use the standard command `setup('C13','CDCl3')` (or whatever solvent is appropriate), the spectrum obtained should be properly referenced. Now even if you can’t see the peaks in the spectrum, you can apply the appropriate knowledge of the expected chemical shift range to place two cursors where you think the edges of that range will be, and use the `movesw` command to narrow the spectral width.

3. Obtain a proton spectrum using the same cabling you will use for indirect detection (and `s2pulr` if appropriate) and narrow the spectral width (remember, when using `s2pulr` you move `dof` and not `tof`). Check the calibration of the pulse width by entering **`pw=4*pw ga`**. Look only at the signals near the center of the spectrum and see if they produce a null signal. If they are negative, enter **`pw=pw+0.8 ga`**; if they are positive, enter **`pw=pw-0.8 ga`**; repeat until a good null is found, then enter **`pw=pw/4`**.

4. Switch to the HMQC experiment, and set the relevant parameters based on the results of steps 2 and 3.

5. Enter **`phase=1 ni=1 dm='nnn' null=0 ai wexp='wft dssh'`**. Set **`j`** to an appropriate value (normally 140 for C–H), and set **`nt`** to 4 or more transients, depending on the concentration of the sample (signal to noise needs to be sufficient

to allow you to see the ^{13}C satellites). Now set **pwx** to an array of 0 and 90° and enter **au** to acquire two spectra. Proceed only if the two spectra are sufficiently different to give you confidence that the second spectrum is showing you satellite peaks only and not just residual uncanceled intensity of the protons attached to ^{12}C.

If you are convinced that you are correctly connected but not happy with the quality of the spectra achieved at this step, skip ahead to step 8 and optimize the `null` parameter, then return here to check and optimize `pwx`. In either case, this is a good time to go over the checklist in the section "Cancellation Efficiency," page 181, making sure you have done everything possible to optimize cancellation.

The spectrum in Figure 54 shows the result of this experiment on a sample of 1% 3-heptanone at 300 MHz, using `nt=64 null=2.0` and `d1=2`.

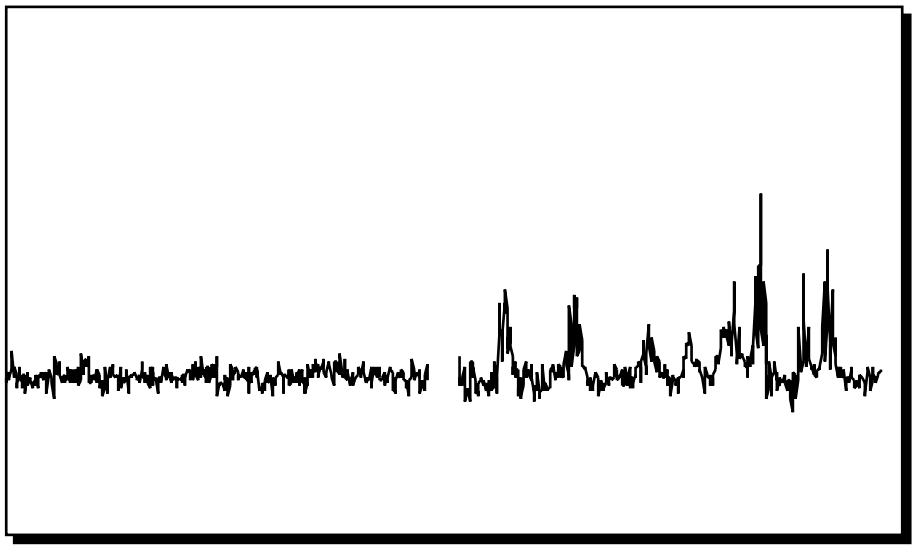


Figure 54. Verifying Cancellation with `pwx=0,90`

6. Now run an array of **pwx** around your expected 90° value, picking the one that gives you the largest satellite signals.
7. If you will be decoupling during acquisition, set **pwx** to its 90° value and enter an array of **dm='nnn','nny'**. These two experiments should show coupled and decoupled spectra, respectively.
8. Now, if appropriate, optimize the parameter `null`. Set **nt=1 ss=4** and enter an array of **null** values with at least one very short value (e.g., 0.001) and one very long value (e.g., 2.0). Because this experiment depends on the relaxation times of the spins involved, you'll also want to set **at** and **d1** to the same values you'll be using in the 2D experiment. Now run the array and select the value of **null** for which either most of the peaks, or the biggest peaks, or the peaks you are most interested in (the criterion is up to you), are approximately zero; remember, no one value of `null` will be correct for all peaks. Figure 55 shows this experiment run on a sample of 28 mg of gramicidin, with `null` arrayed over the range of values: 0.001, 0.05,0.1,0.2,0.3,0.4,0.5, and 2.0; examination of the spectra shows clearly how different values of `null` might be chosen.
9. If presaturation is desired (`hmqc` only, and not on *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*), you can set it up in the following manner. We will need to observe

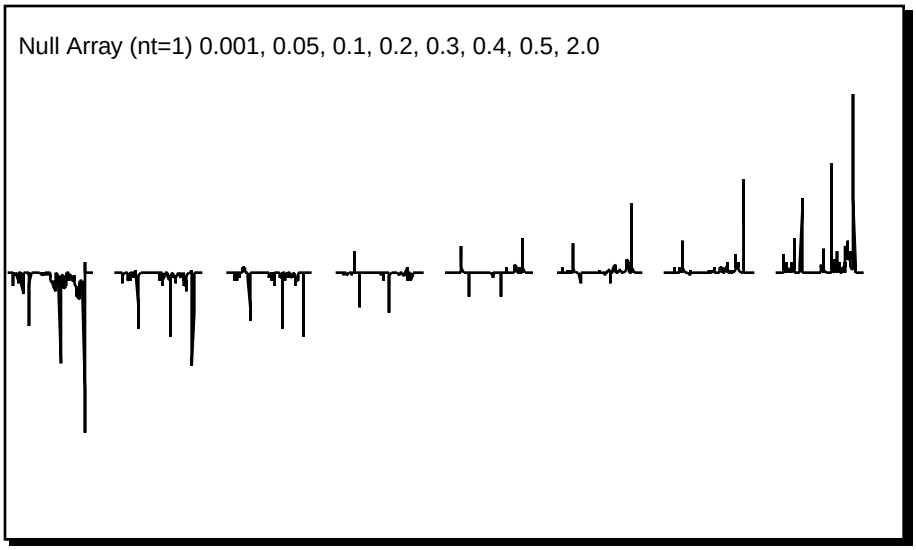


Figure 55. Optimizing the BIRD Nulling Time

the ^{12}C-bound protons, so set **nt=1 dm='nnn'**, and set **null=0** to omit the nulling period (for now at least).

a. Enter **ga** and a proton spectrum will be observed.

b. Move the FID to a different experiment, join the different experiment, and re-transform the data (e.g., **mf(1,2) jexp2 wft**).

c. Set the cursor on a peak that is to be removed by presaturation, and enter **nl movetof**. Note the value of tof selected and then copy this value back to your original experiment into the parameter satfrq (e.g., **jexp1 satfrq=x**).

d. Now set **satflg='yn'** and **satdly** equal to a time significant compared with T_1 of the peaks (e.g., satdly=1).

e. Array **satpwr** to find the minimum value for which the peak will be removed (e.g., satpwr=10,7,4,1 au). When this is determined, if you wish to use presaturation, set **satflg='yy'**, reset **null**, and set **satpwr** to the value determined. If you do not wish to use presaturation, set **satflg='nn'**.

10. Set up the 2D experiment. Set **ni** between 128 and 256, **phase=1,2**, and **nt** to an appropriate number (comparable to what you were using in step 5).

11. Phasing in f_2 is accomplished by performing a 1D transform on the first increment with **wft(1)** and phasing the spectrum, paying attention only to the ^{13}C satellite peaks. In f_1, the combination of the usually large spectral width and the pulse in the center of the evolution time produces large negative values for lp1.

    Reasonably good starting points for the f_1 phase can be calculated according to the following formulas:

    lp1= –sw1 × 360° × ((2×rof1)+(2 × pw)+(4 × pwx/π) (for hmqcr)
    lp1= –sw1 × 360° × ((4 × pwx)/π) (for hmqc)
    rp = –lp1/2

Table 34 lists typical parameters for a quick 2D experiment on the 1% 3-heptanone sample on UNITY and VXR-S systems. Expect to see artifacts in these spectra. The residual

uncanceled signals from protons attached to ^{12}C show up as stripes parallel to the f_1 axis at the frequency of each ^{1}H peak. This artifacts will be larger for peaks with long T_1, such as solvent peaks (e.g., residual protons on a deuterated solvent) or methyl groups. In Figure 56 they are seen at 2.4 ppm, 1.0 ppm, and 0.9 ppm.

Table 34. Parameter Values for HMQCR on Natural Abundance Sample

Parameter	*300 MHz*	*400 MHz*
Proton spectral width	1000	1000
Carbon spectral width	4000	4000
Number of transients (`nt`)	16	16
`tof`	–5732.9	–7060.7
`dof`	–598.6	–1761.1
Number of increments (`ni`)	32	32
Mode	Hypercomplex (`phase=1,2`)	Hypercomplex (`phase=1,2`)
`d1`	2.0	2.0
`null`	2.0	2.0
`at`	0.256	0.256
`gf`	0.128	0.128
`gf1`	0.008	0.008
Solvent	$CDCl_3$	$CDCl_3$
`j`	130	130
`lp1`	–125.2	–125.2
`rp1`	62.6	62.6

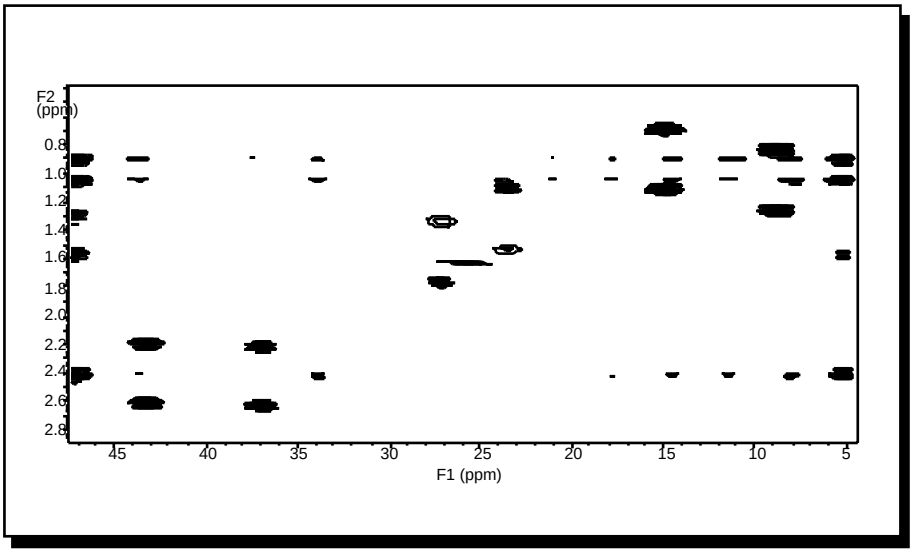


Figure 56. Coupled HMQC Spectrum of 3-Heptanone

Axial peak artifacts, which are common, will show up either at f_1=0 (the center of the spectrum in f_1) or, as in Figure 56, at the edges of the spectrum in f_1 (if FAD is used), again

at f_2 frequencies corresponding to each ^1H peak and possibly through the entire spectrum. Another common artifact seen in Figure 56 is the “0,0” artifact in the exact center of the spectrum. Some peaks in the ^1H spectrum, of course, will not appear in the HMQC spectrum, because they represent protons that are not bound to ^{13}C (e.g., protons from water or NH groups). This is not the case with 3-heptanone, however.

If you are unfamiliar with HMQC spectra, you may be surprised to see multiplet structures. You should realize that during the detection period we are detecting a normal (i.e., with ^1H-^1H couplings) proton spectrum, albeit a spectrum of only those protons attached to ^{13}C. Thus, in Figure 57, we see that the proton attached to the carbon at 37.2 ppm is a quartet (it’s adjacent to a CH_3 group), while the proton attached to the carbon at 43.4 ppm is a triplet (it’s adjacent to a CH_2 group). In the ^1H spectrum itself, these two groups of protons are heavily overlapped (see Figure 58).

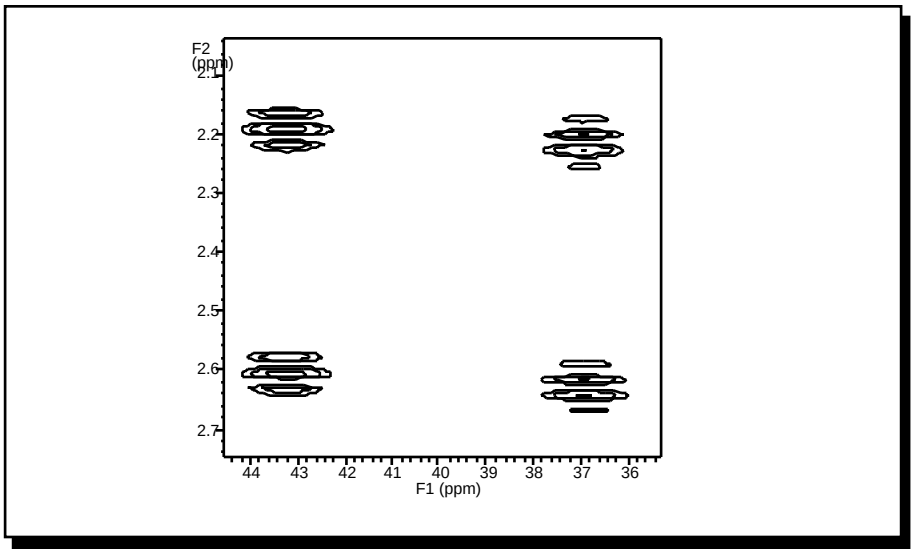


Figure 57. Expansion of Coupled 3-Heptanone HMQC Showing Multiplets

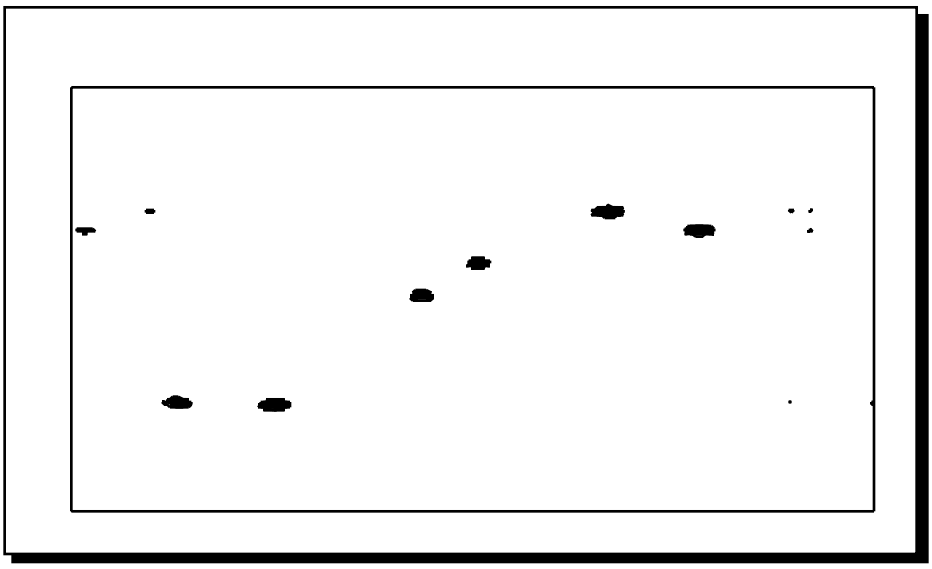


Figure 58. Decoupled HMQC Spectrum of 3-Heptanone

5.15 Differences for ^{15}N Indirect Detection

Calibrations and operations for ^{15}N proceed largely along the lines outlined above for ^{13}C. In the standard sample, 2% ^{15}N-benzamide (Part No. 00-968120-97), the ^{15}N satellite lines are partially obscured by other resonances in the conventional 1D spectrum, and so the ^{15}N pulse width calibration must be done using multi-transient HMQC experiments as described in "Typical Experimental Protocol for HMQC Experiments," page 194. Be sure to use a J appropriate for NH couplings (90 Hz) in this case.

A step that can often be done in ^{15}N work of peptides is to make sure that the γB_2 is sufficient to decouple the relatively narrow range of ^{15}N chemical shifts expected in such samples but no more. This minimizes heating effects and improves cancellation. Typical acquisition times (`at`) are 0.075 to 0.1 seconds.

5.16 HSQC Experiment

The `hsqc` macro sets up parameters for the HSQC pulse sequence, a heteronuclear Overbodenhousen experiment using REVINEPT.

Applicability

Not supplied with *MERCURY-VX*, *MERCURY*, and *GEMINI 2000* systems.

Parameters

`sspul='y'` selects for *trim(x)-trim(y)* sequence at the start of the pulse sequence; `sspul='n'` selects a normal experiment.

`satmode='yn'` gives presaturation during relaxation period (`satdly`) with the transmitter; `satmode='nn'` gives no presaturation during relaxation period (`satdly`); `satmode='ny'` gives presaturation during only the null period.

`satfrq` sets the presaturation frequency.

`satdly` sets the saturation time during the relaxation period.

`satpwr` sets the saturation power for all periods of presaturation with xmtr.

`hs='yn'` sets a homospoil pulse (`hst`) during the d1 relaxation delay.

`null` is the delay associated with the BIRD nulling.

`tpwr` is the power level for ^{1}H transmitter pulses.

`pw` is a 90° transmitter pulse length for protons (the observed nucleus).

`pwxlvl` is the power level for X decoupler pulses.

`pwx` is a 90° decoupler pulse length for X.

`jxh` is a one-bond heteronuclear coupling constant to X (in Hz).

`phase=1,2` for hypercomplex experiment with F1 quadrature (complex F1-FT).

Chapter 6. Data Analysis

Sections in this chapter:

- 6.1 “Spin Simulation,” this page
- 6.2 “Deconvolution,” page 207
- 6.3 “Reference Deconvolution,” page 210
- 6.4 “Addition and Subtraction of Data,” page 213
- 6.5 “Regression Analysis,” page 218
- 6.6 “Chemical Shift Analysis,” page 224

6.1 Spin Simulation

VNMR software includes an iterative spin simulation program based on the FORTRAN program LAME, also known as LAOCOON with magnetic equivalence added. LAME calculates the theoretical spectrum for spin-1/2 nuclei, given the chemical shifts and the coupling constants.

Up to eight closely coupled, non-equivalent spins (ABCDEFGH) can be handled. Equivalent spins can be treated by magnetic equivalence factoring to extend the simulation to systems such as A3B2CD3. The X-approximation can be used to handle different types of nuclei. Nuclei are treated as different types if there is at least one spare letter in the alphabet between their groups (e.g., ABD and ABX are both systems using the X-approximation.) Frequencies, intensities, energy levels and transitions can be listed, and simulated spectra can be displayed and plotted.

Parameters can be adjusted by iteration to approach a given experimental spectrum. For iterative runs, one or several parameters can be kept constant. In addition, one or several parameters can be set equal to each other and held equal during the course of the iteration.

To acquaint yourself with the spin simulation software, we suggest you work through the next section, “Spin Simulation Step-by-Step.” Note how the regression menus simplify the procedure. A number of specialized commands and parameters are also available. Table 35 lists these commands and parameters.

For more information about the spin simulation algorithms, refer to the following:

- Bothner-by, A.A. and Castellano, S., *J. Chem. Phys.*, **41**, 3863 (1964).
- Emsley, Feeney, and Sutcliffe, eds. 1966. *Progress in Nuclear Magnetic Resonance Spectroscopy*, Vol.1, Chap. 3. Oxford: Pergamon Press.
- Stanley, R.M.; Marquardt, D.W.; and Ferguson, R.C., *J. Chem. Phys.*, **41**, 2087 (1964).

Table 35. Spin Simulation Commands and Parameters

Commands	
`assign*`	Assign transitions to experimental lines
`cla`	Clear all line assignments
`dga`	Display group of spin simulation parameters
`dla<('long')>`	Display spin simulation parameters arrays
`dlalong`	Long display of spin simulation parameter arrays
`dll*`	Display listed line frequencies and intensities
`dsp<(file)>`	Display calculated spectrum
`initialize_iterate`	Set iterate string to contain relevant parameters
`spinll<('mark')>`	Set up a slfreq array
`spins<(options)>`	Perform spin simulation calculation
`spsm(spin_system)`	Enter spin system
`undospins`	Restore spin system as before last iterative run
`* dll<('pos'<,noise_mult>)><:lines>`	
`assign<('mark')>, assign(transistion_number,line_number)`	
Parameters	
`cla` {array of real values}	Calculated transition number
`clamp` {array of real values}	Calculated transition amplitude
`clfreq` {real values}	Calculated transition frequency
`clindex` {array of real values}	Index of experimental frequency of a transition
`iterate` {string of parameters}	Parameters to be iterated
`niter` {1 to 9999}	Number of iterations
`slfreq` {real values}	Measured line frequencies
`slw` {0.01 to 1e6}	Spin simulation linewidth
`smaxf` {–1e10 to 1e10}	Maximum frequency of any transition
`sminf` {–1e10 to 1e10}	Minimum frequency of any transition
`sth` {0 to 1.00}	Minimum intensity threshold
`svs` {0 to 1e10}	Spin simulation vertical scale

Spin Simulation Step-by-Step

The simplest way to get acquainted with the spin simulation software is to work through a step-by-step example. The following example is complete with comments to help you understand what you are doing at each step.

1. Click on **Main Menu** > **File** > **Set Directory** > **Parent**.

   The text window displays a list of directories (entries with a backslash as the last character in the name) and files (if any). The status window (near the top of the screen) displays the pathname of the current directory.

2. Click on **Parent** as many times as necessary until the status window displays the message: `Directory now "/".`

3. Click the mouse on the directory entry `vnmr/` in the text window until it turns to inverse video. Then click on the **Change** button.

   The text window displays the list of subdirectories and files (if any) in `vnmr`.

4. Click the mouse on the directory entry `fidlib/` in the text window until it turns to inverse video. Then click on the **Change** button.

   The text window displays the list of subdirectories and files (if any) in `fidlib`.

5. Click the mouse on the directory `fid1d.fid/` until it turns to inverse video.

6. Click on **Return** > **Load** > **Process** > **Transform**.

   The graphics windows displays a spectrum and the opening menu from the interactive spectrum display program (`ds`) appears.

7. Click on **Next** > **Dscale**.

   A scale is displayed under the spectrum.

8. Click the left mouse button near 3.5 ppm and the right mouse button near 3.2. Then click on **Expand**.

   The six-line pattern shown will be simulated as an AX2Y system.

9. Click on **Th**.

   A horizontal line for the threshold appears.

10. Use the left mouse button to move the threshold line below the tops of the peaks.

11. Enter **`dll`**.

    The text window displays a line listing, which will be used later.

With a spectrum now set up, you are ready to use the spin simulation menus to display a simulated spectrum.

1. Click on **Main Menu** > **Analyze** > **Simulation**.

   You are now in the Spin Simulation Main Menu.

2. Click on **Spin System** > **other** > **other** > **AX2Y**.

   This picks the spin system and initializes its parameters.

3. Click on **Set Params**.

   The spectrum reappears.

4. Click the left button in the center of the six-line pattern and enter **`A=cr`**.

   This sets the chemical shift of spin A to the position of the cursor.

5. Click the left button on the center of the left-most line, the right button in the center of the second left-most line, and enter **`JAY=delta.`**

   This sets the JAY coupling constant to match the difference frequency.

6. Click the right button on the center of the third line, and enter **`JAX=delta`**. Then click on **Return**.

7. Click on **Show Params**.

   This confirms your entry of the spin system parameters.

8. Click on **Simulate**.

   After a brief moment, the simulated spectrum will appear.

If you want to continue with iterative spin-simulation, take the following steps:

1. Enter **`iterate?`**.

   The status window displays `iterate='A,JAX,JAY'`, which confirms that the `iterate` parameter was automatically set.

2. Click on **assign** > **auto assign**.

   The `assign` macro is executed, which assigns the lines from the `dll` listing to the lines from the previous simulation.

3. Click on **iterate**.

This performs an iterative optimization and displays the resulting spectrum.

4. Click on **list**.

   The listing contains the values of the A, JAX, and JAY parameters that give the best iterated fit to the experimental spectrum.

Spin Simulation Menus

Almost the entire spin simulation analysis is available through six menus:

- Spin Simulation Main Menu

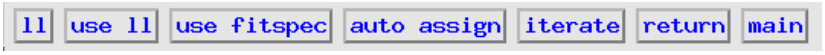
- Spin Simulation First Menu

- Spin Simulation Second Definition Menu

- Spin Simulation Third Definition Menu

- Spin Simulation Secondary Menu

- Spin Simulation Line Assignment Menu



The Spin Simulation Main menu is the opening menu. Each of the other menus is entered through buttons on this menu or submenus of this menu. To open the Spin Simulation Main menu, click on Main Menu > Analyze > Simulation.

Entering a Spin System

The command `spsm(spin_system)` enables creating a spin system as an alphanumeric string of upper-case letters, and creates and initializes appropriate parameters to describe the various chemical shifts and coupling constants. Chemical shifts are stored in parameters A through Z, and the coupling constants are stored in the parameters starting with JAB and ending with JYZ. Different nucleus types are handled by using letters starting with A for the first type, X for the second, and M for the third.

Spin Simulation Parameters

Spin simulation commonly uses the following global parameters:

- `cla` and `clfreq` together make up a table consisting of line numbers assigned by the spin simulation program and the corresponding frequency of a measured line when the intensity of the line is above a threshold value set by the parameter `th`.

- `clamp` stores the transition amplitude of calculated transitions when they are above a threshold set by parameter `sth`.
- `clindex` is an index of experimental frequency of a transition.
- `slfreq` is a list of measured line frequencies.
- `sminf` and `smaxf` are the minimum and maximum frequency limits for calculation of the final simulated spectrum. These should be set before the calculation is performed. If the Set Params button is used, `sminf` is initialized to `sp`, and `smaxf` is initialized to `sp+wp`.
- `sth` is the minimum intensity threshold above which transitions are listed and included in the simulated spectrum. A typical value is 0.05.
- `svs` is the maximum intensity of calculated transitions.

The command `dga` displays the file of simulation parameters.

Performing a Spin Simulation

The command `spins` performs a spin simulation, using the current spin system parameters. The following variations are available:

- `spins('calculate','energy')` puts an energy level table in the output file.
- `spins('calculate','transitions')` puts a second table of transitions ordered by transition number in the output file.
- `spins('iterate')` runs in an iterative mode to match experimental and calculated lines.
- `spins('iterate','iteration')` lists parameters after each iteration in the output file.

The output file is `spins.list` in the current experiment. This file always includes the calculated transitions ordered by frequency.

Using the current table of transitions and intensities, `dsp<(file)>` calculates the simulated spectrum (using the current value of parameter `slw` for the linewidth) and displays the spectrum. `dsp` can only be used after the `spins` program has been run. When `dsp` has a filename as an argument, the spectral information is taken from that file. After the display appears, it may be modified and plotted like any other 1D spectrum.

Iterative Mode

The following commands are used to set up files for the “iterative” mode of spin simulation in which the calculated spectrum approximates an experimental spectrum.

- `spins('iterate')` performs the simulation in the iterative mode.
- `initialize_iterate` selects a default value for the parameter `iterate` that will cause all parameters to be iterated. `iterate` is a string parameter that contains a list of parameters (separated by commas) to be iterated during iterative spin simulations. Typical value is `'A,B,JAB'`. If the Set Params button is used, `iterate` is initialized to a string containing parameters appropriate to the current spin system. The parameter `niter` is the maximum number of iterations that will be used by an iterative simulation. If the Set Params button is used, `niter` is initialized to 20.
- `cla` clears the file of line assignments used for iteration (matching simulated spectra to actual data). `dla` displays this file.

- `dlalong` stores the line assignments in the file `spini.la` of the current experiment. This command is most useful in more complex problems where the text window is too small for the `dla` display. `dlalong` displays the file in the text window.
- `spinll` copies the list of frequencies from the last line listing by `nll` or `dll` (contained in the parameter `llfrq`) into the simulation line frequency parameter `slfreq`. `spinll` also clears the previous line assignments and runs `dla`.
- `spinll('mark')` places the line positions in the file `mark1d.out` into the parameter `slfreq`. This is useful if you want to manually assign the lines.
- `assign` without an argument assigns the nearest calculated transitions to the lines from a `dll` or `nll` listing after `spinll` has placed them in `slfreq`. For positive lines only, use `dll('pos')`. All lines may not be assigned and transitions must be greater than `sth`, which should be 0.05 or greater to prevent assignment of extremely small lines. `assign` can also be run by clicking on the auto assign button.
- `assign('mark')` is the same as assign except that the file `mark1d.out` is used instead of the `dll` listing. Use the cursor and the mark button to place the lines to be assigned in the `mark1d.out` file. This file is cleared by `mark('reset')`. Use `nl` to move the cursor to the center of a selected line.
- `assign(t#,l#)` assigns a single calculated transition number (`t#`) to a line from a `dll` listing (the index is `l#`). `assign(t#,0)` removes the calculated transition assignment.
- `undospins` restores a spin system as it was before the last iterative run. Chemical shifts, coupling constants, and transition assignments are returned to those existing immediately before an iterative spin simulation.

The experimental line listing for `assign` should be made from the part of the spectrum whose limits are `sminf` and `smaxf`. If a frequency in `slfreq` has already been assigned a line number, a new entry will be made with the same frequency. If the line assignments produced by `assign` needs to be modified, refer to the description of the `spins` command in the *VNMR Command and Parameter Reference* for details.

The Spin Simulation Line Assignment menu is especially useful for iterative spin simulation. However, individual assignments are made using the `assign` command as previously described.

Spin Simulation Files

The `spins.list` file is an output table made by the spin simulation program. This file can be displayed by clicking on the list button. The following files can exist in the current experiment but, except for the file `spini.la`, are not normally of interest when spin simulation is run from VNMR:

- `spini.la` is the current transition assignments for an iterative spin simulation (produced by the `dlalong` command).
- `spini.savela` is the transition assignments for iterative spin simulation in a format readable by the macro `undospins`.
- `spini.outpar` are the values of the chemical shifts and coupling constants after an iterative spin simulation.
- `spini.indata` is the line assignment input for the UNIX-level program `spins` (deleted by `spins` after iterations are completed).
- `spini.inpar` is a list of parameters whose values are to be determined by `spins('iterate')`.

- `spins.inpar` is a list of initial settings of a number of spin simulation parameters.
- `spins.outdata` is a file of frequencies, amplitudes, and transition numbers from a spin simulation. It is used in calculating the displayed spectrum.
- `spins.stat` contains constants related to iteration (deleted by the `spins` program).

6.2 Deconvolution

The VNMR software allows the deconvolution of observed spectra into individual Lorentzian and/or Gaussian lines. Up to 2048 data points from an expansion of an experimental spectrum can be deconvoluted at one time, and up to 25 lines can be fit to this section of the observed spectrum. For each line, the line shape can be defined to be Lorentzian, Gaussian, or a combination of both.

The following parameters are available for each line:

- Frequency (in Hz) of line
- Intensity of line
- Linewidth (in Hz) at half-height of line
- Gaussian fraction of line: 0.0 (completely Lorentzian) to 1.0 (completely Gaussian)

All parameters can be fit at the same time, or selected parameters can be removed from the fit. In addition, a linear baseline correction is always added to the fit to avoid large errors produced by base line offsets.

Deconvolution accomplishes much of its work by means of text files, which are written into the user's current experiment directory:

- `fitspec.inpar` contains the starting parameters (frequency, intensity, linewidth, and Gaussian fraction) for a subsequent fitting operation.
- `fitspec.indata` contains the point-by-point intensity of the spectrum in the region of interest (the region that is displayed when the fitting is begun).
- `fitspec.outpar` contains the final parameters (frequency, intensity, linewidth, and Gaussian fraction) after a fit has been done.
- `mark1d.out` contains the result of a `mark` operation during a spectral display. By using the Use Mark button, this file may be used as an alternative to the last line list in setting up initial guesses for a fitting operation.

For best results, use the following settings:

- Use `fn` at least 2*`np` if not larger for adequate digitalization of the line shapes.
- For complex problems, use the macro `usemark` (see the description below) to set the best possible initial guesses.

Deconvolution Step-by-Step

1. Retrieve a sample 1D FID by entering `rt('/vnmr/fidlib/fid1d')`.
2. Enter `fn=65000`.

   For proper digitalization of the line shape, you should almost always use a larger Fourier transform size than "normal" when the spectrum is to be deconvoluted.
3. Transform the FID by entering `wft`.

4. Using standard spectral manipulation operations, expand the two-line pattern near 8.0 ppm until it fills the center third of the display, with baseline on both sides. Enter `ai` (or click on buttons to select the `ai` mode) to select the absolute intensity mode, which is always required for deconvolutions, simulations, etc. Set a threshold that lists exactly two lines, then enter `dll`.

5. Click on Main Menu > Analyze > Deconvolution.

   The Deconvolution Menu is displayed.

6. Click on Use Line List.

   This produces a line list and a file containing the starting point for the deconvolution. This button automatically measures the linewidth of the tallest line on the screen and uses that as the starting linewidth for the calculation.

7. Click on Fit.

   The analysis is performed. This particular example is a 6 parameter fit (2 frequencies, 2 intensities, and 2 linewidths). When the analysis is done, the calculated spectrum is displayed in the graphics window, and the numerical results appear in the text window (click on Flip as necessary to alternate between the two). The numerical output should be similar to this:

```
Number of data points:      404
Final chi square:           318046.844
Total number of iterations: 19
Successful iterations:      19
Digital resolution          0.365 Hz/point
ITERATION HAS CONVERGED
Parameters:
line frequency intensity integral linewidth gaussian fraction
1    3176.780  125.293    423.948   2.154       0.000*
2    3168.700  135.451    421.147   1.979       0.000*
```

8. Click on Plot.

   The original spectrum, the calculated spectrum, and each of the component lines is plotted automatically, along with the numerical results of the calculation. At the end of this operation, the original spectrum replaces the calculated one.

9. Click on Show Fit to return to viewing the calculated spectrum. Click on Add/Sub to view the original spectrum simultaneously with the calculated one. Select sub from the menu to view the difference between the two.

Performing Deconvolution

Table 36 lists the commands and parameters associated with deconvolution.

The parameter `slw` is the starting default linewidth for deconvolution calculations. This linewidth is set automatically when deconvolution is operated using the menu mode and is bypassed if the `usemark` macro has been used in conjunction with two cursor input. Typical value for `slw` is 1.

The command `fitspec` performs spectrum deconvolution by fitting experimental data to Lorentzian and/or Gaussian line shapes. `fitspec` uses as a starting point data in a file `fitspec.inpar`, which must be prepared prior to performing the calculation. This file contains the frequency, intensity, linewidth, and (optionally) the Gaussian fraction of the line shape. Any number followed by an asterisk (*) is held fixed during the calculation; all other parameters are varied to obtain the best fit.

`fitspec` creates a file `fitspec.indata`, which is a text representation of the spectral data (that part of the spectrum between `sp` and `sp+wp`). After the calculation is finished,

Table 36. Deconvolution Commands and Parameters

Commands	
`dsp<(file)>`	Display calculated spectrum
`fitspec<(<'usell'><,><'setfreq'>)>`	Perform spectrum deconvolution (VNMR)
`fitspec`	Perform spectrum deconvolution (UNIX)
`mark*`	Determine intensity of spectrum at a point
`plfit`	Plot deconvolution analysis
`setgauss(fraction),` `setgauss(fraction*)`	Set a Gaussian fraction for line shape
`showfit`	Display numerical results of deconvolution
`usemark`	Use mark as deconvolution starting point

`* mark<(f1_position)><:intensity>,`
`mark<(left_edge,region_width)><:intensity,integral>`
`mark<(f1_position,f2_position)><:intensity>`
`mark<(f1_start,f1_end,f2_start,f2_end)><:intensity,integral,c1,c2>`
`mark<('trace',<options>)><:intensity,integral,c1,c2>,`
`mark('reset')`	

Parameter	
`slw` {0.01 to 1e6}	Spin simulation linewidth

the results of the fit are contained in a file `fitspec.outpar`, with a format identical to `fitspec.inpar`. All lines are set to have a linewidth of `slw`, and a fixed Gaussian fraction of 0. (Refer to the *VNMR Command and Parameter Reference* for information about keyword options available with `fitspec`.)

The `setgauss` macro modifies the output of the last deconvolution (`fitspec.outpar`) and makes it the input for a subsequent analysis (`fitspec.inpar`), after first modifying the Gaussian fraction:

- To allow this fraction to vary, use the format `setgauss(fraction)` where `fraction` is the Gaussian fraction of the line shape, a number naturally limited from 0 to 1, for example, `setgauss(0.4)`.
- To fix the fraction, use the format `setgauss(fraction*)`, suffix the `fraction` value (defined the same as above) with an asterisk and enclose the value in single quotes, for example, `setgauss('1.0*')`.

In some cases it will not be possible to produce a line list that is a suitable starting point for a deconvolution. In this case, or in any case, the results of using the Mark button during a previous spectral display (`ds` program) may be used to provide a starting point. If the mark has been made with a single cursor, the information in the file `mark1d.out` contains only a frequency and intensity, and the starting linewidth is taken from the parameter `slw`. If the mark is made with two cursors, placed symmetrically about the center of each line at the half-height point, `mark1d.out` will contain two frequencies, an intensity and an integral. In this case, the starting frequency is taken as the average of the two cursor positions; the starting linewidth is taken as their difference.

Display and Plotting

After a deconvolution, the results are written into a file `fitspec.outpar` in an abbreviated format. The macro `showfit` converts these data to an output format more suitable for examination and printing.

The command `dsp('fitspec.outpar')` displays the theoretical spectrum described by the parameters produced by a deconvolution calculation. The macro `plfit` produces a

complete output plot of a deconvolution analysis, plotting the observed spectrum, the full calculated spectrum, each individual component, as well as the numerical results of the analysis. It can be invoked with the Plot button in the Deconvolution menu.

Deconvolution Menu

All actions necessary for deconvolution are accessible from the Deconvolution Menu.

This menu is opened by clicking on Main Menu > Analyze > Deconvolution. The Deconvolution Menu can also be opened by the command `menu('fitspec')`.

6.3 Reference Deconvolution

The `fiddle` program performs reference deconvolution, using a reference signal with known characteristics to correct instrumental errors in experimental 1D or 2D spectra. The main command to start the program can take multiple string and numeric arguments:
`fiddle(option<,file><,option<,file>><,start><,finish><,increment>)`

`option` can be the following keywords:

`'alternate'`	Alternate reference phase + / – (for phase sensitive gradient 2D data).
`'autophase'`	Automatically adjust phase.
`'displaycf'`	Stop at the display of the correction function.
`'fittedbaseline'`	Use cubic spline baseline correction defined by the choice of integral regions.
`'invert'`	Invert the corrected difference spectrum/spectra.
`'noaph'`	Do not automatically adjust zero order phase of the reference region.
`'nodc'`	Do not use dc correction of the reference region.
`'nohilbert'`	Do not use Hilbert transform algorithm; use the extrapolated dispersion mode reference signal unless `'noextrap'` is also used as an option.
`'normalise'`	Keep the corrected spectrum integrals equal to that of the first spectrum.
`'readcf'`	Read the correction function from `file`; the argument `file` must immediately follow `'readcf'`.
`'satellites'`	Use the satellites defined in `file` in the ideal reference region; `file` should be in `/vnmr/satellites`.
`'stop1'`	Stop at the display of the experimental reference FID.
`'stop2'`	Stop at the display of the correction function.
`'stop3'`	Stop at the display of the corrected FID.
`'stop4'`	Stop at the display of the first corrected FID.
`'verbose'`	Show information about processing in the main window.
`'writecf'`	Write the correction function to `file`; the argument `file` must immediately follow `'writecf'`.
`'writefid'`	Write out the corrected FID to `file`; if '`file` does not begin with `/` it is assumed to be in the current working directory.

Table 37 list other commands for 1D and 2D variations of the `fiddle` program.

Table 37. Reference Deconvolution Commands

Commands	
`fiddle*`	Perform reference deconvolution
`fiddled*`	Perform reference deconvolution subtracting alternate FIDs
`fiddleu*`	Perform reference deconvolution subtracting successive FIDs from first
`fiddle2d*`	Perform 2D reference deconvolution
`fiddle2D*`	Perform 2D reference deconvolution
`fiddle2dd*`	Perform 2D reference deconvolution subtracting alternate FIDs
`fiddle2Dd*`	Perform 2D reference deconvolution subtracting alternate FIDs
`* (option<,file><,option<,file>><,start><,finish><,increment>)`	

Reference Deconvolution of 1D Spectra

Only spectra that contain a well-resolved reference signal dominated by a single component (i.e. not a simple multiplet) are suitable for reference deconvolution.

1. Fourier transform the raw FID with **`ft`**, preferably having zero filled (i.e. set `fn` >= 2*`np`). (If there are sinc wiggles, use `wft` with `gf` = `at`*0.6.)

2. Set the reference line to the chosen signal using the **`rl`** command, and then use two cursors on either side of the line to define a region of spectrum that includes all of the reference signal plus a little clear baseline but no other signals. This reference region will be used to define the instrumental line shape.

3. Decide what line shape you would like to convert the instrumental line shape to, and set the weighting parameters accordingly. Thus, if you want a 1-Hz wide Lorentzian, set **`lb`** to 1 and all other weighting parameters to `'n'`.

   Bear in mind the signal-to-noise ratio penalty for resolution enhancement: if the experimental line is 2 Hz wide and you set `lb=0`, you get an infinitely sharp line with infinitely poor signal-to-noise. For most purposes, a sensible strategy is to set `lb` to *minus* the expected *natural* linewidth, and choose `gf` to give reasonable S/N; this strategy should convert the instrumental line shape to Gaussian. Where the signals of interest are broader than those of the reference, resolution enhancement can easily be obtained by making `lb` more negative.

4. Enter the **`fiddle`** command to carry out the reference deconvolution and display the corrected spectrum. The integral should remain unchanged, so any resolution enhancement will result in an increase in the amplitude of both signal and noise.

5. To save the corrected data, use the option `'writefid'` when doing the reference deconvolution. For example, to store the file `correctedfid.fid` in the current working directory, enter `fiddle('writefid','correctedfid')`.

   The options `'writecf'<,file>` and `'readcf'<,file>` respectively write and read the correction function. Therefore, when you perform reference deconvolution on one FID using `fiddle` with the `'writecf'` option and then use `fiddle` with `'readcf'` to process another FID, the first correction function corrects the second FID. Reference deconvolution can be useful for heteronuclear lineshape correction (provided that the spectral widths for the two nuclei are in the ratio of the respective magnetogyric ratios) or for correcting spectra in which a reference signal has been suppressed (e.g., you could correct an INADEQUATE spectrum for lineshape errors by using a correction function derived from the normal carbon spectrum).

To correct a series of spectra in an arrayed or 2D experiment, use numeric arguments, as with `ft`: `fiddle(1)` corrects spectrum 1, `fiddle(2,3)` spectra 2 and 3, etc.

Many reference signals have satellites. Like the familiar one-bond ^{13}C satellites, for example, TMS has singlet satellite signals from coupling to ^{29}Si and quartet satellites (normally unresolved) from three-bond coupling to ^{13}C. For most purposes, ^{13}C satellites are small enough to be ignored, but where high accuracy is required or there are stronger (e.g. ^{29}Si) satellites, satellite signals can be included in the specified form of the ideal reference signal by invoking the `'satellites'` option.

The `/vnmr/satellites` directory contains the file `TMS` with details of the TMS satellite signals. The command `fiddle('satellites','TMS')` allows for the satellite signals when deconvoluting using TMS as a reference.

The format for satellite files is that each line in the file consists of three real numbers in the following order:

- Separation of the satellite line from the parent signal, in Hz (0.5 JXH in the absence of homonuclear coupling).
- Intensity relative to the parent signal (natural abundance divided by the number of satellite lines [usually 2]).
- Isotope shift to high field, in ppm.

For example, the line

```
3.3     0.023     0.001
```

would correspond to a pair of satellite signals from a spin-1/2 isotope with an abundance of 4.6%, a coupling to the observed nucleus of 6.6 Hz, and an isotope shift to high field of 0.001 ppm.

Multiple lines in the satellite file account for multiple satellite signals on the parent peak.

To perform corrected-difference spectroscopy, use the command `fiddled` to produce the corrected difference between successive spectra, which divides `arraydim` in half. The difference spectrum is written into the second element of the pair. Because the main aim of reference deconvolution here is to optimize the purity of the difference spectrum, the target line shape would normally be chosen to give the best possible S/N; this method corresponds to choosing a target line shape approximately twice the width of the raw experimental signals of interest. The command `fiddleu` produces corrected differences between successive FIDs and the first FID.

Reference Deconvolution of 2D Spectra

The commands `fiddle2d`, `fiddle2D`, `fiddle2dd`, and `fiddle2Dd` function in just the same way as the parent `fiddle` program. Because the principal objective in 2D reference deconvolution is usually the reduction of t1-noise, ideal line shape parameters are normally chosen for optimum S/N ratio rather than resolution enhancement.

To perform 2D reference deconvolution:

1. Choose **`fn`** (preferably with `fn`>=2*`np`) and **`fn1`**.
2. Enter **`ft`** to transform the raw data (as mentioned earlier, if there is significant signal left at the end of `at`, it might be necessary to use `wft` with `gf` set).
3. Display the first increment with **`ds(1)`**, adjust the phase of the reference signal, and use **`rl`** to select the reference signal.

   In earlier versions of `fiddle`, it was necessary to create a parameter, `phinc`, to anticipate the changes in the reference signal phase with increasing evolution time.

The current algorithm automatically adjusts the phase (unless you select the `'noaph'` option). Deconvolution will set the reference signal phase as a function of `t1` to place the reference signal at frequency `rfp1` in `f1`. Therefore, remember to set `rfl1` and `rfp1` before using `fiddle2D` or the `f1` frequencies might unexpectedly change.

4. Define the reference region with the two cursors, and then enter the command **`fiddle2D('writefid',<file>)`** (or `fiddle2Dd` if a 2D difference spectrum is required, as with corrected HMBC). The `'writefid'` option is essential, because `fiddle2D` alone does not store the corrected time-domain data. If phase-sensitive gradient-enhanced 2D data is to be processed, alternate FIDs will have opposite phase modulations (i.e., the experimental array will alternate N-type and P-type pathways); in such a case, use the `'alternate'` option.

After deconvolution is complete, the corrected 2D FID data can be read into an experiment and processed as normal (although if `fiddle2Dd` has been used, `arraydim` no longer matches the arrays set and it might be necessary to set the arguments to `wft2d` explicitly rather than using `wft2da`).

References

Further information on reference deconvolution can be found in the following literature:

Taquin, J. *Rev. Physique App.* **1979**, *14***,** 669.

Morris, G. A. *J. Magn. Reson.* **1988**, *80***,** 547.

Morris, G. A.; Cowburn, D. *MRC* **1989**, *27***,** 1085.

Morris, G.A., Barjat, H., Horne T.J., *Prog. NMR Spectrosc.***1997**, *31*, 197.

Gibbs, A; Morris, G. A. *J. Magn. Reson.* **1991**, *91***,** 77.

Gibbs, A.; Morris, G. A.; Swanson, A.; Cowburn, D. *J. Magn. Reson.* **1983,** *101*, 351–356.

Rutledge, D. N. Ed. *Signal Treatment and Signal Analysis in NMR*, Chapter 16. Elsevier Science, 1996.

6.4 Addition and Subtraction of Data

The process of addition and subtraction of data is implemented using experiment 5 (`exp5`) as the add/subtract buffer file (overwriting any data that may have existed in `exp5`). Tools available for working with the add/subtract experiment include:

- Add/Subtract menu.
- Noninteractive commands.
- Interactive add/subtract program.

Table 38 lists the commands and parameters used with the add/subtract experiment.

Add/Subtract Menu

The Add/Subtract menu in the main menu system provides choices that activate command for clearing the add/subtract experiment (`clradd` command), activating an interactive

Table 38. Add/Subtract Experiment Commands and Parameters

Commands	
`add*`	Add current FID to add/subtract experiment
`addi`	Start interactive add/subtract mode
`clradd`	Clear add/subtract experiment
`jexp1, ..., jexp9999`	Join existing experiment
`select*`	Select a spectrum or 2D plane without displaying it
`setvalue*`	Set value of a parameter in a tree
`spadd*`	Add current spectrum to add/subtract experiment
`spmin`	Take minimum of two spectra in add/subtract experiment
`spsub*`	Subtract current spectrum from add/subtract experiment
`sub*`	Subtract current FID from add/subtract experiment

`* add<(multiplier<,'new'>)>, add('new'), add('trace',index)`
`select<('next'|'prev'|selection)><:index>,`
`select<(<'f1f3'|'f2f3'|'f1f2'><,'proj'><,'next'|'prev'|plane>)><:i>`
`setvalue(parameter,value<,index><,tree>)`
`spadd<(multiplier<,shift>)>, spadd('new'), spadd('trace',index)`
`spsub<(multiplier<,shift>)>, spsub('new'), spsub('trace',index)`
`sub<(multiplier<,'new'>)>, sub('new'), sub('trace',index)`	

Parameters	
`arraydim` {number}	Dimension of experiment
`lsfid` {'n',–fn/2 to ni or fn/2}	Number of complex points to left-shift `np` FID
`phfid` {'n',–360 to 360, in deg}	Zero-order phasing constant for `np` FID

spectral add/subtract mode (`addi`), performing noninteractive spectral addition (`spadd`) and subtraction (`spsub`), and taking the minimum of two spectra (`spmin`):

Typical entry into this menu is by clicking on Main Menu > Analyze > Add/Sub. The menu can also be opened by entering the command `menu('addsub')`. The commands activated by the buttons are described in more detail in the next section.

Noninteractive Add/Subtract

The buffer used as for the add/subtract experiment (`exp5`) is first cleared using `clradd`, then different FIDs or spectra are added to or subtracted from the accumulating total by the commands `add`, `sub`, `spadd`, and `spsub`.

Adding and Subtracting FIDs

The `add` and `sub` commands add and subtract the last displayed or selected FID to and from the contents of the add/subtract experiment, respectively. An optional argument allows the FID to first be multiplied by a multiplier (the default is 1.0). The parameters `lsfid` and `phfid` can be used to shift or phase rotate the selected FID before it is combined with the data in the add/subtract experiment.

A multi-FID add/subtract experiment with FIDs 1 and 2 can be created with the `add` or `sub` command using the `'new'` keyword as follows:

1. Create the add/subtract experiment with a single FID by entering the following commands from some experiment:
   `clradd select(1) add`

2. Make the add/subtract experiment contain an array of two FIDs corresponding to the original FIDs 1 and 2 by entering:
   `select(2) add('new')`
   If you had entered `select(2) add` in step 2, a single FID that is the sum of the original FIDs 1 and 2 would have been made instead of an array.

The `arraydim` parameter may need to be updated after constructing a multi-FID add/subtract experiment. To do this, join `exp5` by entering `jexp5` and then enter `setvalue('arraydim',numFIDs,'processed')`

where `numFIDs` is the number of FIDs in that experiment. (e.g., if 12 FIDs were put into `exp5`, enter `setvalue('arraydim',12,'processed')`).

Individual FIDs in a multi-FID add/subtract experiment can be added to and subtracted from. The `add` and `sub` commands without a `'trace'` keyword adds or subtracts from the first FID in the add/subtract experiment. Adding the `'trace'` keyword followed by a required index number selects another FID to be the target of the add/subtract.

For example, `select(4) add('trace',6)` takes the fourth FID from the current experiment and adds it to the sixth FID in the add/subtract experiment.

When using the `'trace'` keyword, that FID must already exist in the add/subtract experiment by using an appropriate number of `add('new')` or `sub('new')` commands.

Adding and Subtracting Spectra

Noninteractive spectral addition and subtraction uses the `spadd` and `spsub` commands. The last displayed or selected spectrum is added to (`spadd`) or subtracted from (`spsub`) the current contents of the add/subtract experiment.

Each spectrum can be optionally multiplied and shifted using the `multiplier` and `shift` arguments, respectively. For example, entering `spadd(0.75,10)` multiplies the spectrum by 0.75 and shifts the spectrum by 10 to the left. A positive value of `shift` shifts the spectrum being added or subtracted to higher frequency, or to the left. A negative value of `shift` shifts the spectrum being added or subtracted to lower frequency, or to the right. To shift a spectrum without multiplying it, use a multiplier of 1.0.

A multi-element add/subtract experiment can be created with the `spadd` or `spsub` command. Using the keyword `'new'` creates a new spectrum in the add/subtract experiment, for example:

1. Create the add/subtract experiment with a single spectrum in it by entering **`clradd select(1) spadd`** from some experiment.
2. Enter **`select(2) spadd('new')`** to make the add/subtract experiment contain an array of two spectra corresponding to the original spectra 1 and 2, respectively.
   If you instead entered `select(2) spadd`, a single spectrum that is the sum of the original spectra 1 and 2 would have been made instead of an array.

Individual spectra in a multi-element add/subtract experiment can subsequently be added to and subtracted from. The `spadd` and `spsub` command without a `'trace'` keyword adds to or subtracts from the first spectrum in the add/subtract experiment. Adding the `'trace'` keyword followed by a required index number selects another spectrum to be the target of the add/subtract. For example, `select(4) spadd('trace',6)` takes the fourth spectrum from the current experiment and adds it to the sixth spectrum in the add/subtract experiment.

When using the `'trace'` argument, the indexed spectrum must already exist in the add/subtract experiment by using an appropriate number of `spadd('new')` or `spsub('new')` commands. The results can be examined by joining experiment 5 (`jexp5`) and using the normal spectral display (e.g., `ds`) and plotting commands.

Interactive Add/Subtract

Interactive add/subtract provides a convenient way to manipulate two different spectra. A "spectrum" may be a 1D spectrum, a trace from a 2D spectrum, or even a spin simulated spectrum. Both horizontal displacement and vertical scale of the two spectra to be added or subtracted are under interactive control. If the spectra can be phased, they can be phased independently. The result can be manipulated using any of the standard software (e.g., the command `pl`), including further interactive add/subtract with another data set.

Interactive add/subtract is a multi-step process using the command `addi`. The same as noninteractive add/subtract, interactive add/subtract uses `exp5` as an add/subtract buffer file, so no important data should be present in that experiment when you begin the process.

To Start Interactive Add/Subtract

Interactive add/subtract starts exactly the same as noninteractive add/subtract:

1. Enter **`clradd`** to clear the buffer.
2. Enter **`spadd`** to add in the current spectrum as the starting point.
3. Enter **`addi`** to start the interactive add/subtract program. The `addi` program can also be opened by clicking on the Interactive Mode button in the Add/Subtract Menu or by clicking on the Add/Sub button in the Deconvolution Menu.

Upon opening the `addi` program, a second spectrum is selected and the interactive process is started.

A screen display appears similar to Figure 59.

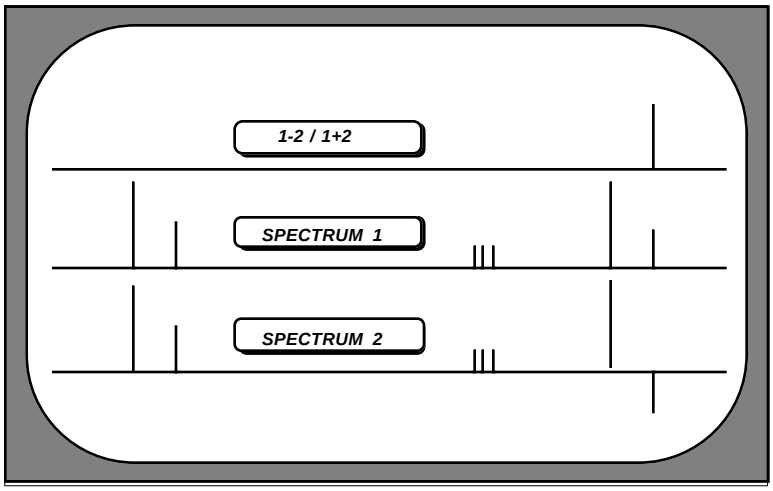


Figure 59. Interactive Add/Subtract Display (addi Program)

Spectrum 1, the spectrum selected by the `spadd` command, appears in the center of the display. Spectrum 2, the spectrum which was active when `addi` was typed, appears on the bottom. The sum or difference of these spectra appears on top of the screen; when `addi` is

first entered, this spectrum is the sum (1 + 2) by default. The Interactive Add/Subtract menu (described in the next section) also appears.

Displayed at the bottom of the screen is the name of the currently active spectrum (the interactive one). Also displayed there is the current result mode (add, sub, or min).

Interactive Add/Subtract Menu

The Interactive Add/Subtract Menu has the following buttons (the labels on some buttons change depending on what mode you are in):

Each button functions as follows:

Box	The first button is labeled Box or Cursor. When Box appears, you are in the cursor mode, and clicking on this button changes you to the box mode with two cursors.
Cursor	When Cursor appears, you are in the box mode, and clicking on this button changes you to the cursor mode with one cursor.
Select	Selects whether the current, add/sub, or result spectrum is active.
Expand	The third button is labeled Expand or Full, depending on whether you are in the box or cursor mode. When Expand appears, you are in the box mode, and clicking on this button expands the area between the cursors.
Full	When Full appears, you are in the cursor mode, and clicking on this button displays the full area.
sp wp	Adjusts the start and width of the active spectrum.
sub	The fifth button is labeled sub, min, or add. When sub appears, clicking on the button makes the result spectrum to be the difference between the current and the add/sub spectra.
min	When min appears, clicking on the button makes the result spectrum to be a minimum intensity of either the current or the add/sub spectra.
add	When add appears, clicking the button makes the result spectrum to be a sum of the current and the add/sub spectra.
save	Saves the result spectrum in the add/sub experiment and returns to the last menu.
return	Returns to the last menu without saving the result.

This menu is not user-programmable.

To Manipulate Spectra

Manipulation of the spectra is performed using the mouse:

- Left button positions the cursor or pair of cursors. If the sp wp button is selected, the left mouse button adjusts the start of the display.
- Center button changes the vertical scale of the spectrum so that it goes through the current mouse position. If the mouse is positioned at the left edge of the spectrum, the horizontal position of the spectrum is adjusted.

- Right button positions the second cursor relative to the first cursor. If the sp wp button is selected, the right mouse button adjusts the width of the display.

The important points to understand are few. The Select button is used to toggle between different modes of control. When the label at the bottom of the screen reads "`active`: `current`," all of the parameters (except `wp`) control spectrum 2, and spectrum 2 can be phased, scaled, or shifted relative to spectrum 1.

After clicking on the Select button, the label at the bottom of the screen reads "`active`: `addsub`," and all of the parameters except `wp` control spectrum 1.

Clicking on Select again toggles the label to read "`active`: `result`," so now parameter changes affect only the sum or difference spectrum. Note that `wp` always controls all spectra, since differential expansions of the two spectra are not supported. Note also that the colors of the labels change to match the colors of the different spectra.

The sum/difference spectrum displayed on the screen while `addi` is active is strictly a temporary display. Once all manipulations have been performed, and assuming the sum/difference is something you wish to perform further operations with (such as plotting), it must be saved into the add/subtract buffer (`exp5`) by clicking on the save button. At this point spectrum 1, which was in the add/subtract buffer, will be overwritten by the sum or difference spectrum, and `addi` will cease operation.

In most cases, you enter `jexp5 ds` to display the difference spectrum on the screen, ready for further manipulation (expansion, line listing, etc.) and plotting. If you wish to continue with the add/subtract process by adding in a third spectrum, display that spectrum in the usual way and enter `addi` again.

6.5 Regression Analysis

The process of establishing correlations between two or more variables is called *regression analysis* or *correlation analysis*. The established regression or correlation can then be used to predict one variable in terms of the others. Often, paired data indicate that a regression may have a certain functional form, but we do not want to make assumptions about any underlying probability distributions of the data.

This type of problem is often handled by the least squares curve-fitting method. Specific examples of this were used for the analysis of T_1 and T_2 NMR data and for the analysis of kinetics data. Also available within VNMR are tools for fitting arbitrary data to selected functional forms.

The regression process in VNMR takes a set of data pairs from the file `regression.inp` and attempts to fit a curve to the set. The implemented curves are first, second, and third order polynomials and an exponential in the form:

```
y = a1 * exp(-x/tau) + a3
```

There are further possibilities as the original data may be displayed against a choice of linear, squared, or logarithmic scales.

Regression Commands and Menus

Table 39 lists the commands associated with regression analysis.

To remove the need to determine the correct arguments for the commands involved, especially `expl` and `analyze`, regression can be performed almost completely through the following menus.

Table 39. Regression Commands

Commands	
`analyze*`	Generalized curve fitting in regression mode
`autoscale`	Resume autoscaling after limits set by scalelimits
`expfit*`	Make least-squares fit to exp. or poly. curve (UNIX)
`expl<(<options,>line1,...>)>`	Display exponential or polynomial curves
`pexpl<(<options,>line1,...>)>`	Plot exponential or polynomial curves
`poly0`	Display mean of data in regression.inp file
`rinput`	Input data for regression analysis
`scalelimits*`	Set limits for scales in regression

```
* analyze('expfit',xarray<,option,option,...>)
  expfit options <analyze >analyze.list
  scalelimits(x_start,x_end,y_start,y_end)
```

Regression 1 menu:

Regression 2 menu:

Regression 3 menu:

These menus are accessible by selecting Analyze in the Main Menu, and then selecting Regrs from the Analyze Menu.

The actions of the buttons 1 through 6 on each menu are described below. The next button in the Regression 1 selects the Regression 2 menu, the next button in the Regression 2 selects the Regression 3 menu, selects the next regression menu. The Return button in the Regression 3 menu returns to the Regression 1 menu.

Regression Analysis Step-by-Step

1. Write and save the text file `regression.inp` that contains the data pairs you wish to analyze. The next section describes the format of this file. Create it by one of the following methods:
   - Use the macro `rinput`. This program displays a series of prompts requesting the axis label titles and the data pairs. When you finish, `rinput` creates the file `regression.inp` in the correct format. `rinput` does not allow you to edit the data; correct errors using a text editor after you complete the program.
   - Use a text editor such as `vi` or `textedit`.
   - Create a MAGICAL II macro for this purpose.
2. Enter the command **`expl('regression')`**. Alternatively, select any one of the buttons x-linear, y-linear, x-square, y-square, x-log, and y-log. Each of the buttons also scales for displays and plots either the x or y axis as labeled on the button. In the case of multiple data sets in the input file, data sets may be selected with the command `expl('regression1',line#,line#...)`.

The `expl` command uses the values in the `regression.inp` file to display a graph of the data points. It also creates the files `analyze.inp` (needed by `analyze` to run the analysis) and `expl.out` (display information for `expl`).

You might also want to use the `poly0` macro to calculate and display (as horizontal lines) the mean of the data in the file `regression.inp`.

3. Run the **`analyze('expfit','regression',option,'list')`** command, where `option` is `'poly1'`, `'poly2'`, `'poly3'`, or `'exp'`, and then enter **`expl`** to see the results as a graph. Alternatively, click on one of the buttons **dp-linear**, **dp-quad**, **dp-cubic**, or **dp-exp**. These buttons include displaying the results using `expl`.

   `analyze('expfit','regression',option,'list')` calls the UNIX program `expfit`, which creates the files `analyze.out` (used by `expl` to display the results) and `analyze.list` (a table of results). The type of fitting is determined by `analyze` option or button you provide:

Fitting	*analyze option*	*Button*
linear	`'poly1'`	dp-linear
quadratic	`'poly2'`	dp-quad
cubic	`'poly3'`	dp-cubic
exponential curve	`'exp'`	dp-exp

The menu system allows immediate display of the regression results and selection of another type of regression if results are not satisfactory. Figure 60 shows quadratic fittings for the data given in the example of the `regression.inp` file in the next section.

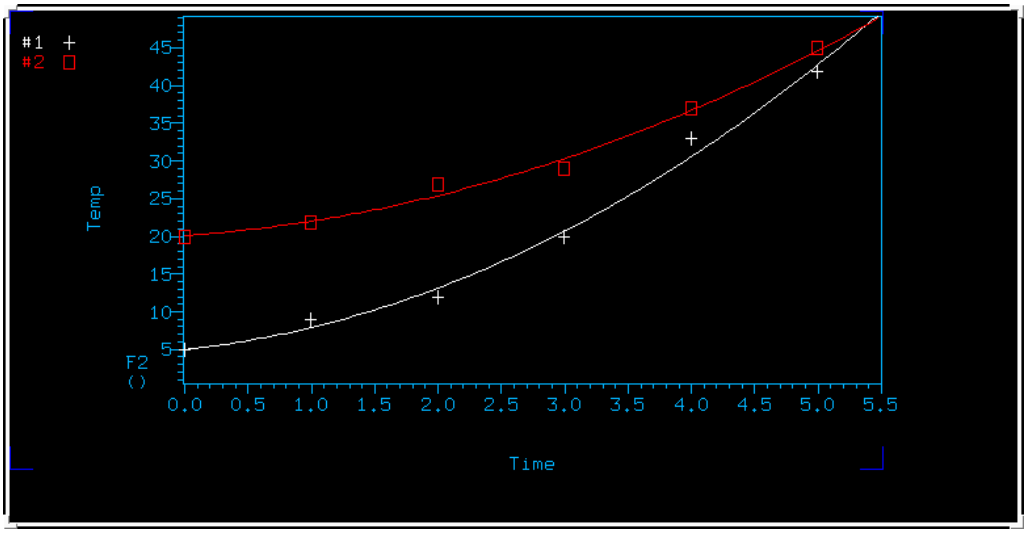


Figure 60. Display of Regression Fittings (expl Program)

4. To plot the analysis, select the **plot** button in the Regression 3 menu or enter **`pexpl page`**. To plot selected lines, enter **`pexpl(index#,index#,...)`**.

   Because `expl` and `pexpl` set the scale automatically to show all points (if possible), you may want to enter the **`scalelimits`** macro to set limits for the scales. Entering `scalelimits` with no argument starts an interactive process in

which you are prompted for the four scale limits. You can also enter the limits for the x-axis and y-axis as arguments: `scalelimits(x_start,x_finish, y_start,y_finish)`. The limits are retained as long as an `expl` display is retained. To return to automatic scaling by `expl`, enter **`autoscale`**.

5. To show the results of the analysis in the text window, select the **dp output** button from the Regression 3 menu or enter **`cat(curexp+'/analyze.list')`**.

Contents of "analyze.out" File

The data input file is `analyze.out`, except for regression when the input file is `regression.inp`. The file `expl.out` saves certain display and plot parameters.

Values can be 2048 points maximum from a data set, 2048 points maximum from all sets displayed/plotted, 8 data sets maximum displayed, and 128 data sets maximum are read.

The following is an example of `analyze.out`. Numbers ❶, ❷, etc. identify lines in the example and are not part of the actual file:

```
❶ exp 7 regression
❷ D1 C0 C1 C2
❸     1  5   linear linear
❹ Exponential Data Analysis
     time
     amp
❺    NEXT  5
❻ 1  -248.962  22.8025  226.157
❼       2        4
         3        9
         4       16
         5       25
         6       36
```

The following is a description of the numbered parts of this file:

❶ The keyword `exp` is followed by a number for a curve type from Table 40.

Table 40. Curve Types.

Type	*Function*	*Functional Form*
0	T_1/T_2	*(a0 – a2)*exp(–t/a1) + a2*
1	Increasing kinetics	*a0*exp(–t/a1) + a2*
2	Decreasing kinetics	*–a0*exp(–t/a1) + a2 + a0*
3	Diffusion	*a0*exp(–D1*uu) + a2*exp(–a1*D1*uu)* where $uu=C0+C1*t+C2*t^2$
4	None	No theoretical curve (use `'link'`)
5	Linear	*a0 + a1*t*
6	Quadratic	$a0 + a1*t + a2*t^2$
7	Exponential	*a0 * exp(–t/a1) + a2*
8	Contact time	*(a3 –(a3 – a0)*exp(–t/a1)) *exp(–t/a2) + a0*
9	Cubic	$a0 + a1*t + a2*t^2 + a3*t^3$

The keyword `regression`, if present, indicates regression output

❷ Floating point constants *D1*, *C0*, *C1*, and *C2*, if present, are used only with the diffusion function (curve type 3).

❸ An integer for the number of data sets (curves), followed by an integer for the number of data point pairs in the set. For regression, the words are scale types for the *x* and *y* axes: `linear`, `square`, and `log`.

❹ Title line. Use `No Title` when a title is not desired. Two additional text lines for the *x* and *y* axis titles are present in output from regression.

❺ The keyword `NEXT` identifies the start of a data set, and the integers that follow give the number of data point pairs in the data set.

❻ The first integer specifies the number (usually 1) of the data point symbol used for the data set. The next three integers are the coefficients *a0*, *a1*, and *a2* (see Table 40) and must all be present, even for functions that do not use all three (e.g., first-order polynomial). If a particular number is not appropriate, put any number there. *a3* must be also be present for cubic and contact time functions (curve types 7 and 8).

❼ Data point pairs in the set.

Next is an example of `regression.inp` file for generalized curve fitting:

```
❶ time
❷ amp
❸     0       0
❹     NEXT
❺      2.000000  4.000000
        3.000000  9.000000
        4.000000  16.000000
        5.000000  25.000000
        6.000000  36.000000
```

Description of this example:

❶ Line with text for *x*-axis label displayed by `expl('regression')`.

❷ Line with text for *y*-axis label (line must not be too long, usually less than 20 characters). The first non-blank character must not be a digit.

❸ Line containing an integer for the number of data sets followed by another integer for the number of pairs per data set. Both values are `0` if the number of pairs is variable.

❹ A line beginning with the keyword `NEXT` is inserted at the start of each data set when the number of pairs per peak is variable.

❺ The data pairs, listed one pair to a line.

The final example uses the `'file'` argument to the `expl` command:

```
❶ exp  4
❷   1     5
❸   time
❹ 1  0  0  0
❺   2     4
     3     9
     4    16
     5    25
     6    36
```

Description of this example:

❶ Keyword `exp` followed by curve type number.

❷ Number of data sets, followed by number of data point pairs.

❸ Title.

❹ Data point symbol number, followed by three coefficients.

❺ Data point pairs in the set.

Contents of "regression.inp" File

The data input text file `regression.inp` contains a listing of axis labels and data pairs.The data file can contain up to 128 data sets. Data sets are selected by `expl` indexes (up to 6, depending upon length of data sets) with a default to the beginning data sets. The analysis is limited to 1024 data points, with the first part of larger data sets selected.

The following is an example of a `regression.inp` file that shows the format used. Numbers ❶, ❷, etc. identify lines in the example and are not part of the actual file:

```
❶ time
❷ temp
❸   0   0
❹ NEXT
❺ 0.000000    5.000000
   1.000000    9.000000
   2.000000    12.000000
   3.000000    20.000000
   4.000000    33.000000
   5.000000    42.000000
❹ NEXT
❺ 0.000000    20.000000
   1.000000    22.000000
   2.000000    27.000000
   3.000000    29.000000
   4.000000    37.000000
   5.000000    45.000000
```

Description of this file:

❶ X-axis label for display by `expl('regression')`. The label is optional. If used, the first non-blank character in the label must not be a digit.

❷ Y-axis label for display by `expl('regression')`. The label is optional. If used, it must be less than about 20 characters and the first non-blank character in the label must not be a digit.

❸ If the number of pairs per data set is fixed, this line contains an integer for the number of data sets, followed by another integer for the number of data pairs per data set. If the number of pairs per data set is variable (as in this example), both integers are set equal to 0.

❹ If the number of pairs per data set is variable, a line with the word `NEXT` is inserted at the start of each data set.

❺ Data pairs, one to a line, are listed for each data set, in this order:
first pair of first set
second pair of first set
third pair of first set
. . .
first pair of second set

second pair of second set
. . .

6.6 Chemical Shift Analysis

Chemical shifts can be analyzed using the commands `pcss` and `do_pcss` listed in Table 41. When you use `pcss` and `do_pcss`, the list of chemical shifts that is created is saved in the file `pcss.outpar`.

Table 41. Chemical Shift Analysis Commands

Commands	
`do_pcss`	Calculate proton chemical shifts spectrum
`pcss`	Calculate and show proton chemical shifts spectrum

Chapter 7. Pulse Analysis

Sections in this chapter:

- 7.1 “Pulse Shape Analysis,” this page
- 7.2 “Pandora’s Box,” page 230

7.1 Pulse Shape Analysis

The `pulsetool` program is designed to display and examine shaped rf pulses. The standard pulse template file format is the same as for shaped pulses in `/vnmr/shapelib`. Data points are listed as `phase amplitude time-count`, where `phase` is in degrees, `amplitude` is a value between 0 and 1023, and `time-count` is an integer which describes the relative time duration of the step. The program is started by entering the command `pulsetool` in a UNIX window. Table 42 summarizes the command and parameters associated with pulse shape analysis.

Table 42. Pulse Shape Analysis Commands and Parameters

Command	
`pulsetool <-shape filepath>`	RF pulse shape analysis (UNIX)
Parameters	
`phi`	Amount of rotation about the Z-axis
`theta`	Declination relative to XY-plane

The `amplitude` and `phase` are displayed in the small windows at the top of the display, along with the effective frequency of the pulse, the quadrature components of the pulse, and its Fourier transform. You can select the contents of any of the smaller windows for display in the large graphics window in the center of the screen.

Between the small windows at the top of the display and the large central, graphics window is the control panel, home to a number of buttons that perform various operations or activate the routines described below.

Below the main graphics window is a panel that contains miscellaneous information about the current pulse and display status. The directory file name, pulse name, vertical scale, and vertical reference fields display current information that can be altered by the user.

The Steps, Fourier size, Power factor, and Integral fields are advisory only, and may not be entered or changed by the user. Power factor is calculated when a pulse is loaded, and is the mean square amplitude of the pulse. A square pulse has a power factor of 1. The integral of

the pulse is an attempt to calculate the tip-angle per unit time and B_1 field strength. This number is strictly valid only for pulses that are modulated in amplitude only and can be used to determine the B_1 field required to obtain, for example, a 90° tip with a sinc pulse. To do this, divide the desired tip angle (in revolutions) by the product of the integral value and the pulse length (in milliseconds). The result is the required B_1 field strength, in kHz.

The directory system may be viewed, and pulse files selected for loading through the use of the Files button.

Simulation of the actual response to a pulse, based on Bloch equation calculations, is available by selecting the Simulation button.

A number of standard pulses can be created, with attributes tailored through the Create utility. The data currently displayed in the main graphics window can be saved with the Save button.

Directory and File Operations

In selecting files, both the working directory and the pulse template file name can be specified by direct entry into the Directory and Pulse name fields found in the panel at the bottom of the display (use the Delete key to erase characters, if necessary, and type in the desired name, followed by Return to indicate completion). When the Pulse name field is selected, pressing Return causes the named file to be loaded and displayed.

Alternatively, the Files button causes a popup window to be displayed, listing the contents of the current directory. A trailing slash "/" following a member of the list indicates a subdirectory, and an asterisk "*" an executable file. The Load, Chdir, and Edit buttons operate on an item selected from this listing with the left mouse button:

The Load button causes the selected file to be read, and displayed in the graphics windows. If the file does not correspond to the proper format for pulse template files, an error message is displayed. Comment lines beginning with the pound character "#" are ignored. Descriptive information about the pulse is displayed in the bottom panel—the name of the file, the number of steps in the pulse, the Fourier size required to do the FFT of the pulse, and a "power factor" calculated for the pulse. The power factor is based on the mean square amplitude of the pulse.

The Chdir button changes to and then lists the selected directory.

The Parent button changes to and then lists the parent of the current directory.

The Save button located in the main control panel can be used to save data currently displayed in the main graphics window to a file. When this button is selected, a second button labeled Done appears, along with a type-in field that holds the name of the file that will be created. First, enter an appropriate name, then select the Save button once again to write the file. Once you have entered the Save mode, you can repeat this as many times as you like— display a different attribute in the main window, enter a new file name, and select Save. To exit from this mode, select Done.

The Print button located in the main control panel can create a file that can be used to print the main graphics window on a PostScript printer. When this button is selected, a second button labeled Done appears, along with a type-in field that holds the name of the file that will be created. Selecting the Print button with a appropriate file name in the type-in field writes the file. The file can subsequently be sent to a PostScript printer with the UNIX `lp` command.

Attribute Selection

The six small graphics windows at the top of the tool initially display the different attributes of the current pulse:

- Amplitude
- Phase
- Effective off-resonance frequency
- Real and imaginary quadrature components
- Fourier transform

Any of these six windows can be displayed in the large graphics window by clicking in the appropriate small window with either the left or middle mouse buttons:

- The left mouse button causes the large window to be cleared before drawing and sets the clear mode to on.
- The middle mouse button turns off the clear mode and displays the selected attribute, overlaying any current display in the large graphics window.

Repeated selection of the small Fourier transform window will result in the large window cycling through the magnitude of the Fourier transform, the real component, and the imaginary component.

Scale and Reference

The vertical scale can be adjusted either by clicking the middle button inside the boundary of the large graphics window or by manually entering a value in the Vertical scale field of the bottom panel, ending by pressing Return. Using the middle mouse button causes the scale to be adjusted interactively so that the active curve passes through the mouse arrow. Note that no rescaling occurs if the y-value specified with the middle button does not have the same sign as the actual attribute value at that point on the x-axis. A negative value can, however, be entered as a vertical scale if so desired.

The vertical reference controls the vertical position of the active curve on the large graphics window, representing the offset from zero measured in y-axis units. A positive value moves the curve up, and a negative value moves it down. Like the vertical scale, the vertical reference can be adjusted in one of two ways—a value may be entered manually into the Vertical reference field in the bottom panel, or the middle mouse button can be used interactively anywhere in the large graphics window, while simultaneously holding down the Control key. In the second case, the vertical reference is set so that the curve passes through the mouse arrow.

The vertical scale and reference are reset whenever an attribute is selected from any of the small graphics windows. If things get out of hand, use this by reselecting the current small window with the left mouse button.

Cursors

Interactive left, right, and horizontal cursors are available, and display a readout of position at the bottom of the large window when active. The left cursor is activated by clicking the left mouse button inside the large window. When the left cursor is present, the right cursor can be activated by clicking the right mouse button anywhere to the right of the left cursor. At this point, the right mouse button controls the position of the right cursor independently, while the left mouse button moves both cursors in tandem.

When both cursors are active, the control panel button normally marked Full will read Expand, and can be used to display an expanded view of the region selected between the two cursors. (Note that the clear mode will always be set to on after an Expand or Full operation.) The left and right cursors are turned off by clicking the appropriate mouse button in the large window while simultaneously pressing the Control key.

The horizontal cursor is activated with the Thresh button located in the control panel. When this cursor is active, it is controlled interactively with the middle mouse button. The interactive scale and reference functions normally controlled with the middle mouse are not available when the horizontal cursor is present. Select the Scale button in the control panel to turn off the horizontal cursor and reactivate the scale and reference functions (vertical scale and reference can be adjusted even with the horizontal cursor active by direct entry in the appropriate fields in the bottom panel).

Simulation Overview

The simulation routine simulates the effects of an rf pulse by use of the classical model of nuclear spin evolution described by the Bloch equations. T_1 and T_2 relaxation effects are ignored, in which case the evolution of a magnetization vector in the presence of an applied rf magnetic field can be evaluated by multiplication with a 3 by 3 rotation matrix. The simulation consists of repeated multiplication by such a matrix, whose elements are determined at each step by the values of amplitude and phase found in the pulse template file, and by user input values of initial magnetization, B_1 field strength, pulse length, and resonance offset. The simulation is performed over one of three possible independent variables— resonance offset, B_1 field strength, or time, and is determined by the Sweep cycle in the small button panel.

Simulation Parameters

Select the Simulation button in the control panel to activate the Bloch Simulation subwindow. This window consists of a panel containing all required parameters (the pulse length is taken from the value in the bottom panel of the main window) and a small button panel at the bottom of the window. To change the value of any parameter, select it with the left mouse button, then delete the appropriate characters and enter the desired value from the keyboard. Parameters are updated each time the Go button is selected or when the Steps button is selected with Index equal to zero.

The first three parameters in the left hand column describe the starting values for the magnetization components Mx, My, and Mz, whose vector sum must be less than or equal to 1.

The next three fields change to reflect the state of the Sweep cycle, which can be toggled between B_1, Freq, and Time. When Freq is selected, the first of these fields will read B_{1max}, the value of B_1 at the maximum pulse amplitude. The second and third fields determine the lower and upper off-resonance frequency boundaries. When the Sweep cycle is set to B_1, these three fields are reversed so that the first determines a constant off-resonance frequency and the remaining two determine the lower and upper boundaries of the maximum B_1 amplitude. Selecting Time will yield a display of the magnetization as a function of progression through the pulse, at the frequency and B1 field strength specified by the parameter values displayed. In this last case, the number of steps in the simulation is taken from the number of points in the pulse template and may not be altered externally. To get finer resolution in the simulation, use a pulse template with a greater number of steps.

The Initialize cycle determines if the magnetization is reinitialized to the values of Mx, My, and Mz, or if the simulation uses the values at each point that were the result of the previous simulation. In this way, the effect of a series of pulses can be evaluated by loading the pulse and performing the simulation with Initialize set to Yes, then loading the next pulse, setting Initialize to No, and selecting Go. Any number of pulses can be concatenated in this fashion. This feature works only for Frequency and B_1 sweep, but not Time.

When Time sweep is selected, the results can also be displayed in the form of a projected three-dimensional coordinate system, showing the path of the magnetization over the course of the pulse. This display is obtained by selecting the 3D button after first selecting the Go button. When the 3D display is active, the left mouse button controls the viewing angle from within the canvas region delineated by the blue corner markers. This viewing angle is described by the two parameters `phi` (the amount of rotation about the Z-axis) and `theta` (the declination relative to the XY-plane). A “family” of trajectories can be displayed by first selecting any of the small canvases with the middle mouse button, then selecting the 3D button. Changing either the B_1 field strength or the resonance offset followed by the Go button will result in display of the result without clearing the display. To reactivate the automatic clearing feature, select any of the small canvases with the left mouse button. To see the 3D display drawn in real-time, enter a nonzero integer value in the Time field. The appropriate value depends on the number of steps in the pulse and the type of computer you have. Try a value like 100 for a SPARCstation.

The last parameter in this column determines the number of points at which the simulation will be performed along the y-axis. A larger number will give more detail in the result, but will require proportionally more time to complete.

The Index parameter is a counter that updates the status of the simulation, and cannot be set externally. The value displayed is the number of steps in the pulse template that have been completed.

The Step Inc parameter is used by the Step button, described in the section, “Performing a Simulation,” to control the number of intermediate steps to be calculated.

Performing a Simulation

When you have adjusted the parameters to your liking, you will probably want to select the Go button. This does simulation calculations and then displays the results in the first five small graphics windows, replacing (but not destroying!) the pulse information that was displayed there. The Fourier transform information remains unaffected, so that comparisons can be made between this and the exact simulation results.

All of the display functions described elsewhere are active as well, with the simulation data. Additionally, the original pulse data is still present in the background and can be swapped into view with the Display cycle found in the main control panel.

The Step button offers the ability to view the course of the magnetization at intermediate stages through the pulse. When this function is selected, the next Steps Inc steps of the pulse are simulated, starting at the current value of Index. The intermediate result is then displayed in the normal fashion.

During a Go simulation, a small panel containing a Cancel button will pop into view. Use this to stop the simulation if necessary (there may be some delay between selecting the button and the end of the process; it won't do any good to click on Cancel more than once).

Creating a Pulse

The pulse creation routine currently offers the following pulse types:

Square	Hermite 90	Tan swept inversion
Sinc	Hermite 180	Sin/cos 90
Gaussian	Hyperbolic secant inversion	

A file containing the pulse template for any of these pulses can be created from scratch with this utility. Alternatively, pulses can be created for examination only, using the display capabilities of `pulsetool`. Each pulse is generated with user-definable parameters appropriate for the pulse in question.

When the Create button is selected, a menu of pulse types appears. Hold the right mouse button down on the Create button, select one of the pulses in the resulting menu, and release the mouse button. If you decide you don't like any of the possibilities, move the mouse arrow out of the menu area and release the button. When a pulse type is selected, a small window appears with a brief description of the characteristics of the pulse and a set of changeable attributes whose values you may alter if so desired. The number of steps in the pulse is limited to powers of 2 and can be set by clicking the left mouse button, or by holding the right mouse button down and selecting the desired value from the resulting menu. All other attributes, which vary depending on the pulse type, can be altered from their default values by first selecting the appropriate field with the left mouse button, deleting with the Delete key, and typing in the desired value (pressing Return is not required).

At this point, you may select one of the three buttons at the bottom of the window: Preview, Execute, or Done:

- Preview uses the attributes as they appear on the screen to create a pulse that is loaded internally into `pulsetool`. All `pulsetool` features can then be used to examine and evaluate the new pulse. Any previous pulse information is deleted.
- Execute uses the attributes as they appear on the screen to create a pulse, which is written to a standard UNIX file. The name of the file is taken from the file name field in the Create window and written into the current directory, listed in the Directory field in the bottom panel. If a file of the same name already exists, you are asked to confirm your request. If, for any reason, the program is unable to write to the named file, an error message appears. This is generally symptomatic of not having write permission in the current directory.

Currently, there is no convenient way for a user to add new pulse types to those listed above. Suggestions for those pulse types that should be included in the future are welcomed. However, any user-created shaped pulse may be examined using the Files button.

7.2 Pandora's Box

Pandora's Box (Pbox) software creates shape pattern files for experiments involving shaped rf pulses, composite pulses, decoupling and mixing patterns, adiabatic rf sweep waveforms, and pulsed field gradient shapes. The goal of Pbox is to simplify generation and use of different waveforms in NMR experiments to a level where the user does not need to be an expert in selective excitation. Pbox makes the use of complex waveforms as simple as using ordinary rectangular pulses. Indeed, not only does Pbox provide all the necessary parameters (pulse width, power, `dmf`, `dres`, etc.) when the shape files are created, but this information can be extracted at any time from Pbox shape files by macros or directly within pulse sequences. More than 160 different shapes are available from the Pbox library.

Getting Started

The simplest mode of operation is from Pbox menus:

1. Enter **ds** to display a spectrum.
2. Click on **Pbox**.

The Pbox menu system appears:

These buttons have the following actions:

Wavelib	Selects the `wavelib` directory and provides file system utility for selection of shapes.
90	Prompts for cursors to be placed around the signals to be excited and displays the Pbox menu for the definition of 90° excitation pulse shapes.
180	Prompts for cursors to be placed around the signals to be excited and displays the Pbox menu for definition of 180° inversion/refocusing pulse shapes.
Homo-dec	Prompts for cursors to be placed around the signals to be homodecoupled and displays the Pbox menu for definition of decoupling waveforms.
Het-dec	Displays the Pbox menu for the definition of decoupling waveforms. By default, it assumes ^{1}H decoupling over 10 ppm
Mixing	Displays the Pbox menu for the definition of mixing waveforms. By default, it assumes bandwidth of 10 ppm (^{1}H).
Grad	Displays the Pbox menu for the definition of gradient shapes.
Quit	Exits Pbox and displays the 1D Interactive Display menu.

The following steps are typical for creating an excitation pulse:

1. Click on **Pbox** > **90**.
2. Select an excitation band using cursors.
3. Click on **e-Burp1** > **Close** > **Name** > **Close**.

For help in understanding Pbox menus, click on the Help button.

Calibrating the RF Field

To obtain the pulse calibration numbers in the Pbox output, provide the rf field calibration data `ref_pwr` and `ref_pw90` in the input. If menus are used for the first time in the current experiment, Pbox prompts you to provide the necessary information. Therefore, before waveform creation, make sure the rf field has been calibrated and you know the length of the 90° pulse at a given power level.

If the spectrometer amplifiers are linear, which is standard on Varian NMR spectrometer systems, it does not matter at what power level the rf field is being calibrated. However, for maximum accuracy, do the calibration close to the field used in the experiment. An estimate of the rf field is obtained by providing approximate calibration data and using `cal` as an output file name. No waveform is created in this case, and only the calibration results appear in the output.

Creating Waveforms from Macros

Pbox macros provide useful tools for customizing NMR experiments. The simplest way to create a shape is using the pxshape macro. For example, a single band excitation pulse using the E-BURP-1 shape, covering 400 Hz, and shifted off-resonance by –880 Hz from the carrier frequency (middle of the spectrum) can be created and stored in the `alpha.RF` file as follows:

```
pxshape('eburp1 400.0 -880','alpha.RF')
```

The following steps are necessary to create multiply-selective pulses. If the spectrum of interest is on the screen, use the cursors.

1. Enter **`opx('hadamard.RF')`** to open the `Pbox.inp` file and write the file header.
2. Select an excitation band using cursors.
3. Enter **`selex('rsnob')`**.
4. Select the second excitation band using cursors.
5. Enter **`selex('rsnob')`**.
6. Repeat steps 2 to 5 as many times as needed.
7. Enter **`cpx`** to close the `Pbox.inp` file.
8. Enter **`dshape`** to display the last created shape.

If an experimental spectrum is not available, the following slightly different set of macros are used:

1. Enter **`opx('myshape')`** to open Pbox and provide a file name.
2. Enter **`setwave('sech 400.0 -880.0')`** to select first band at –880 Hz.
3. Enter **`setwave('sech 400.0 1240.0')`** to select second band at 1240 Hz.
4. Enter **`cpx(ref_pw90, ref_pwr)`** to close Pbox.
5. Enter **`dshape`** to display the shape file.

The `pbox_pw` and `pbox_pwr` macros are used to load the parameters of the last created shape file into the current experiment:

```
pbox_pw:selpw
pbox_pwr:selpwr
```

Alternatively, the calibration data is directly retrieved from the shape file provided as an argument to the `pbox_dmf` and `pbox_dres` macros:

```
pbox_dmf('ccdec.DEC'):dmf
pbox_dres('ccdec.DEC'):dres
```

where `ccdec.DEC` is the name of the decoupling shape file.

The excitation profile of shaped pulses is conveniently verified using the Pbox Bloch simulator:

1. Enter **`opx`** to open Pbox.
2. Enter **`setwave('iburp2 400.0 -880')`** to select first band at –880 Hz.
3. Enter **`setwave('iburp2 400.0 1240.0')`** to select second band at 1240 Hz.
4. Enter **`pbox_rst`** to reset par-s and write comments.
5. Enter **`pboxpar('name','test.DEC')`** to define the output file name.

6. Enter **pboxpar('bsim','y')** to activate the Bloch simulator.
7. Enter **cpx** to close Pbox.
8. Enter **dshape** to display the shape file.
9. Enter **dprofile('z')** to display inversion (Mx) profile.

In the vast majority of cases, you don't have to tell Pbox whether you are creating a 90° excitation pulse or 180° inversion pulse, or even whether an .RF, .DEC, or .GRD type of waveform is needed, because this information is stored with the corresponding wave file in the `wavelib` directory. Pbox can be forced to change the waveform type by you simply providing the required extension to the output shape file name. Wave files are modified, if necessary, by copying it into your `wavelib` and editing the text file as required. See "Pbox Macro Reference," page 242, for a more complete description of macros.

Creating Waveforms from UNIX

It is sometimes more convenient to create waveforms from the UNIX shell:

```
> Pbox
```

The name of the output shape file is passed as an argument:

```
> Pbox filename
```

The input data are typically stored in the `Pbox.inp` file in your `vnmrsys/shapelib` directory and are modified using standard text editors. Alternatively, most of the necessary data can be provided as arguments to the `Pbox` command. For example,

```
> Pbox myfile -w 'eburp1 480 -1200' -p 40 -l 104
```

generates an E-BURP-1 excitation pulse covering 480-Hz-wide band and shifted –1200 Hz off-resonance using for calibration 104 µs long pw90 at 40 dB power level and stored in `myfile.RF`. Note that the name of the output shape file is always passed as the first argument.

Several other options are accepted by `Pbox`; for example, `-b` activates the Bloch simulator, `-c` calibrates the waveform without creating a shape file, and `-o` print out the available options. (see "Pbox UNIX Commands," page 258, for further information).

Pbox File System

All the information about the waveform to be created (e.g. calibration data, output file name, excitation band definition) is stored in the `Pbox.inp` text (ASCII) file in the user directory `vnmrsys/shapelib`. This file is generated whenever Pbox menus or macros are used. You can also create it by using one of the standard text editors.

Any shape file can consist of one or several shaped pulses that are combined into a single waveform. Each excitation band is defined by a wave definition string (a string of wave variables enclosed between delimiters {and}). The number of wave definition strings in a single `Pbox.inp` file is unlimited. In order to simplify the input file format, the wave variables are entered without names in a strongly predefined order:

```
sh bw(/pw) ofs st ph fla trev d1 d2 d0 wrap
```

The following list describes each of the variables.

`sh`	Shape name as stored in `wavelib`
`bw(/pw)`	Bandwidth in Hz, or pulsewidth in sec, or both
`ofs`	Offset from transmitter offset or carrier in Hz

st	Spin status (0 for Mz or 1 for Mxy)
ph	Phase or phase cycle
fla	Flip angle
trev	Time reversal flag
d1	Prepulse delay
d2	Postpulse delay
d0	Delay before the pulse
wrap	Wraparound parameter

The order of parameters has been chosen such that the importance of parameters is decreasing and rarely used parameters can be omitted or defaulted by assigning a value of n (not used). The following examples are valid wave definition strings.

{qsneeze}	q-SNEEZE pulse applied on resonance, the pulse length will be internally defaulted to 5 ms.
{G3 800}	G3 pulse covering bandwidth of 800 Hz and applied on-resonance.
{sech 400/0.05 -1200}	50 ms long hyperbolic secant pulse covering 400Hz and shifted off-resonance by –1200 Hz.
{WURST2 2k/5m 12k n t5}	5 ms long WURST-2 decoupling pulse covering 2 kHz and shifted off-resonance by 12 kHz uses t5 phase cycle.
{eburp1 450 0.0 n 180} {eburp1 450 820 1 0.0}	Two E-BURP-1 pulses mixed in a single waveform, both covering 450 Hz wide band. The first pulse is applied on-resonance with a phase of 180 deg. The second pulse is shifted to 820 Hz of-resonance, has zero phase and is a de-excitation pulse (status 1). By default such a pulse is time reversed.

A set of Pbox parameters can be used to define the waveform to be generated. The syntax of the Pbox.inp file is straightforward, parameter=value, for instance, name=myshape.RF, or simply name=myshape. The following list describes Pbox parameters and their default values (see "Pbox VNMR Parameters," page 237, for more details):

name=Pbox	Shape file name, the extension is optional.
type=r	Shape type, r - RF, d - DEC, g - GRD.
dres=9.0	As in VNMR, deg. The default value is stored in wavefile.
steps=200	Minimum number of steps (< 64k). The default value is stored in a wave file.
maxincr=30	Max phase incr, deg (<<180).
attn=i	Attenuation, i (internal), e (external) or d (nearest dB step)
sfrq=0	Spectrometer frequency, MHz.
refofs=0	Reference offset, Hz (/ppm).
sucyc=d	Super Cycle, d (default), n (no), name as in wavelib/supercycles. The default value is stored in wavefile.
reps=2	Amount of reports (0-4).
stepsize=n	Size of a single step (ms).
wrap=0	Wraparound parameter (0-1).
header=y	Shape header, y (yes) n (no) i (imaging).
bsim=n	Bloch simulation, y (yes), n (no), a (add), s (subtract), 200 (time in sec).

T1=n	Relaxation time T1 (sec).
T2=n	Relaxation time T2 (sec).
dcyc=1	Duty cycle (0 - 1).
sw=0	Spectral width (Hz)
ptype=selective	pulse type (for imaging only).

The number and order of input parameters is optional and not important.

You can redefine the internally defaulted Pbox parameters by entering the default values in the .Pbox_globals file.

Parameters describing software and hardware limitations are also pre-defined internally and can be redefined by the user in the .Pbox_globals file that is stored in user's home directory. The following list describes global parameters and their default values.

shdir=$HOME/vnmrsys/shapelib/	default shape directory
wvdir=/vnmr/wavelib	default wave directory
maxst=65500	maximum number of steps in waveform
defnp=100	default number of steps
minpw=0.2	minimum step length, in µs
minpwg=2.0	minimum gradient step length, in µs
drmin=1.0	minimum dres
maxamp=1024.0	maximum amplitude
maxgr=32767.0	maximum gradient amplitude
amres=1.0	amplitude resolution
phres=0.1	phase resolution, in degrees
tmres=0.05	time resolution, in µs
dres=9.0	default dres
maxpwr=63	maximum power level, in dB
minpwr=-16	minimum power level, in dB
maxitr=5	maximum number of iterations
maxdev=2.0	maximum deviation, in percent
cmpr=y	waveform compression
minstps=64	minimum steps in Bloch simulation
pw=0.005	default .RF and .DEC pulse length, in sec
pwg=0.001	default .GRD pulse length, in sec

The parameters of individual shapes—Gaussian, E-BURP-1, or hyperbolic secant pulse, etc.—are stored in the wavelib directory, which has several subdirectories, such as excitation, inversion, refocusing. Every individual shape is defined by a set of parameters that can be grouped in several categories.

Wave definition parameters are the following:

amf	amplitude modulation function
fmf	frequency modulation function
su	default supercycle
fla	default flip angle on resonance
pwbw	pulsewidth to bandwidth product
pwb1	pulsewidth to B1max product
pwsw	pulsewidth to sweepwidth product
adb	adiabaticity on resonance

ofs	offset of excitation bandwidth
dres	default tipangle resolution, in degrees
dash	dash variable
wf	window function
st	default status
dutyc	duty cycle
c1	constant
c2	constant
c3	constant
steps	default number of steps

Wave truncation parameters are the following:

min	minimum truncation threshold (0 to 1)
max	maximum truncation threshold (0 to 1)
left	truncation from left (0 to 1)
right	truncation from right (0 to 1)
cmplx	flag, retain real (1), imag (–1) or complex(0) part of wave
wrap	wraparound factor (0 to 1)
trev	time reversal flag (yes = 1, no = 0)
srev	frequency sweep reversal flag (0 to 1)
stretch	stretching factor (>= 0)
dcflag	dc correction, y/n

Additional parameters are usually data matrices, such as Fourier coefficients or square wave parameters e.g. length, phase, amplitude, etc. These matrices are listed without parameter names. The size of the data matrix given is defined by:

cols	number of columns
rows	number of rows

Pbox incorporates the following amplitude modulation (AM) functions:

sq	square (constant amplitude)
sqa	square wave amplitude modulation (used for “composite” pulses)
gs	Gaussian
lz	Lorentzian
sch	sech (hyperbolic secant)
hta	tanh (hyperbolic tangent)
tra	triangular amplitude (ramp)
sc	sinc function
csp	cosine power
wr	wurst (wideband uniform rate smooth truncation)
sed	seduce-1, mixture of sech and sin
qp	quadrupolar
ata	amplitude mod for CA atan frequency sweep pulse
exa	exponential amplitude
tna	tangential amplitude
fs	Fourier Series
ft	inverse Fourier Transform

Pbox incorporates the following frequency modulation (FM) functions:

`ls`	linear sweep (chirp)
`tns`	tangential sweep (tan)
`ht`	hyperbolic tangent sweep (tanh)
`lzs`	constant adiabaticity Lorentzian sweep
`ca`	constant adiabaticity (CA) sweep (frequency modulated frame)
`cas`	constant adiabaticity sweep (phase modulated frame)
`cs`	cosine / sine pulse frequency sweep
`cs2`	CA cosine square frequency sweep
`ccs`	CA cosine frequency sweep
`sqw`	squarewave phase modulation
`fsw`	frequency switch (step function)
`fslg`	frequency switched as per Lee-Goldburg

Pbox VNMR Parameters

The following list describes Pbox VNMR parameters.

`name` — Name and extension of the output shape file. If the extension is not given, the shape type is set according to the `type` parameter. The default name is internally set as `Pbox`. This can be changed in the `.Pbox_globals` file.

`type` — Shape type. Allowed values are `r` (.RF type), `d` (.DEC) or `g` (.GRD). If the shape type is not defined and the shape file is given without an extension, the shape file type is determined from the wave file according to the following criteria:

- `type` is set to `r` if `pwbw` > 0.0.
- `type` is set to `d` if `dres` > 0.0.
- `type` is set to `g` otherwise.

`dres` — Corresponds to `dres` parameter in VMNR. Active only with .DEC files.

`steps` — Defines the required number of steps in the waveform. The default number of steps is stored with each individual shape in the corresponding wave file. This number can be overridden by Pbox if it is smaller than the internally calculated minimum number of steps, which is necessary to maintain the functionality of the waveform. This number is defined according to the following criteria:

- By the minimum number of steps necessary for adequate representation of the waveform (as in wave file).
- If the waveform is shifted off-resonance, by the Nyquist condition (see `maxincr`).

`maxincr` — Maximum phase increment. By default, set to 30°. This number is active only if the waveform is shifted off-resonance or the shape itself is frequency modulated (e.g., adiabatic sweeps). In order to satisfy the Nyquist condition, `maxincr` should not exceed 180°, otherwise the waveform gets folded back. In fact, the degradation of performance and interference with sidebands can be observed even with a `maxincr` of greater than 90°, but a `maxincr` of less than 90° is recommended.

attn	Fine attenuation mode, which uses the following allowed values: i (Internal), default.Fine attenuation is implemented by internally rescaling the waveform within the amplitude range set by maxamp (0 to 1023). e (External) Fine attenuation is implemented by externally rescaling the waveform using linear modulators. The internal amplitude is set to maxamp (1023.0) and the required fine attenuator setting is produced in the output. d Attenuate to the nearest dB step by changing the pulse width, which will affect the excitation bandwidth typically within 5%, which is tolerable in most applications. The internal amplitude is set to maxamp (1023.0) 4.5i Internally attenuate to a given (4.5 kHz) B1 field strength by adjusting the pulse length. 4.5e Externally attenuate to a given (4.5 kHz) B1 field strength by adjusting the pulse length. 4.5I Internally attenuate, keeping course power level at a given (45 dB) power level. 4.5E Externally attenuate (with fine power), keeping course power level at a given (45 dB) power level. 45d Attenuate to a given (45 dB) power level by changing the pulse width. The internal amplitude is set to maxamp (1023.0).
sfrq	Spectrometer frequency in MHz.
refofs	Reference offset, usually 0.0. Can be specified if the excitation bands are shifted by or referenced to some frequency. Units: Hz, kHz, or ppm (if sfrq is defined).
sucyc	Super cycle. Allowed values are n (no), d (default) or any name of a super cycle stored in the wavelib/supercycles directory. By default, it is internally set to d. Super cycles can be nested by separating the names with a comma, for example, t5,m4 represents 5 step TPG super cycle nested in four step MLEV-4 super cycle.
reps	Defines level of reporting. Allowed values are 0-4: 0=silent, 1=single line, 2=minimum, 3=medium, 4=maximum. The default is 2.
stepsize	The length of a single step in a waveform. The default units are µs. Note that stepsize disables the maxincr parameter.
bscor	Initiates correction for Bloch-Siegert effect in multiple band excitation, inversion or refocusing pulses. Allowed values are y (yes) or n (no, default). Active only if the number of bands is two or more. Reduces the rf interference effects (see M. Steffen, L.M.K. Vanderseypen and I.L.Chuang, Abstracts of the 41st ENC, p. 268, Asilomar 2000).
wrap	Wraparound parameter. It allows wrapping around the waveform. The allowed values are between 0 and 1.0.
header	Shape file header. Allowed values are y (yes, default), n (no shape file header) and i (imaging). Information required for imaging systems is stored in the shape file header.
bsim	Bloch simulator. Performs Bloch simulation for the given waveform at the moment of waveform generation. Allowed values are y (yes), n (no, default), a (add to the previous simulation), s (subtract from the previous simulation) and any positive integer limiting the simulation time in seconds. The default maximum length of simulation is internally set to 60 seconds and can be redefined in the .Pbox_globals file. Note, that Bloch simulator can also be externally activated, e.g., from menus or using the dprofile macro.
T1	Longitudinal relaxation time, T1 in seconds. Can be required by some waveforms (e.g. SLURP pulses). Optional for the Bloch simulation.
T2	Transversal relaxation time T2, in seconds. Can be required by some waveforms (e.g. SLURP pulses). Optional for the Bloch simulation.

dcyc	Duty cycle. Usually required for homonuclear decoupling applications. Only values between 0.0 and 1.0 are active. Outside these boundaries dcyc is reset to 1.0 (default).
sw	Spectral width. If given, the step size of waveform is set equal to the dwell time (1/sw). Recommended for H-H homo-decoupling applications. It also helps to make sure that excitation sidebands are kept outside the spectral window. Also required for Bloch simulation.
ref_pw90	Reference 90° pulse width (in µs) at ref_pwr. Required for calibration of waveforms. If set to 0.0, the maximum B1 field intensity (in kHz) is reported instead of the power setting.
ref_pwr	Reference power level (in dB steps). See ref_pw90.
ptype	Pulse type. Only necessary with imaging header. By default, set to selective.

Wave String Variables

A reminder is given in Pbox.inp files generated by menus and macros because these parameters appear without names. The wave string variables are listed as they appear in the reminder.

sh	Shape name as in wavelib.
bw/pw	Bandwidth and/or pulsewidth. For most waveforms, only one of the two parameters is required. Pbox distinguishes between bw (in Hz), which is always greater than 1.0, and pw (in sec), which is always less than 1.0. It is up to you which of the two parameters to provide for input, because they are mutually related via the pw*bw product, which is stored with each individual shape in wavelib. Some waveforms (e.g., adiabatic sweep pulses) can require both bw and pw. In such cases, both variables can be provided in a single string using the "/" separator. For example, {WURST2 200.0/0.05} denotes a 50-ms long WURST-2 pulse covering 200 -Hz-wide band. Alternatively, units can be used for clarity, e.g., {WURST2 0.2k/50m}. If the sfrq parameter is defined, bandwidth can also be specified in ppm, e.g., {WURST 20p/5m}.
ofs	Offset of the center of the excitation band in Hz with respect to the carrier frequency (middle of the spectrum). Note that if the sfrq spectrometer frequency, (in MHz) is defined, ofs can also be specified in ppm. In order to specify ofs in terms of absolute frequency, the reference offset refofs (i.e., chemical shift value of carrier frequency) must be defined. For instance, {WURST2 20p/5m 170p} sfrq=225.0 refofs=55p.
st	Spin status. Defines whether the waveform is used for excitation (st=0), refocusing (st=0.5) or de-excitation (st=1), which, in turn, defines whether the wave starts with phase defined by ph (st=1), the ph occurs in the middle of the pulse (st=0.5), or the pulse ends with phase ph (status 0). In addition, the waveforms are time reversed if status is 1, as required for proper de-excitation. Undesired time reversal can be undone using the trev parameter. Furthermore, if several waves of different width are generated, they are bound to the beginning (st=1), middle (st=0.5), or end (st=0) of the waveform. The spin status of the first wave is also used by Bloch simulator as the starting magnetization.
ph	Phase in degrees or phase cycle (super cycle). Usually phase is externally set in the pulse program and this parameter is not required. You can also apply any phasecycle (super cycle) from wavelib/supercycles. The difference between this phase cycle and the sucyc parameter is that phase cycling is carried out before waveform mixing and is therefore independent of other Super cycles, whereas sucyc is applied to the final (mixed) waveform. In this way, several waves of different width can be independently phase cycled and use different super cycles.

`fla`	Flip angle, in degrees. Usually, `fla` is defined in the wave file and there are very few applications where intermediate flip angles are required.
`trev`	Time reversal flag (see `st`). Allowed values are `y` (yes) and `n` (no, default).
`d1`	Prepulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
`d2`	Postpulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
`d0`	Pre-`d1` delay, in seconds. Essentially repeats d1. It is used only for convenience, e.g., if internal duty cycle is defined in shape parameters in `wavelib`. If set to `'a'`, the wave is appended to the previous wave.
`wrap`	Wraparound parameter. Can take values between 0 and 1.0.

Creating Waveforms Using Menus

A set of menus is provided for convenience of pulse shaping from the VNMR window:

Pbox	Pbox180	Pbox180a	Pbox180b
Pbox180r	Pbox90	Pbox90a	Pbox90b
PboxDec	PboxDec2	PboxGrad	PboxHoDec
PboxHoDeca	PboxLib	PboxMix	PboxOpt
PboxOpt2	PboxSol	PboxWva	PboxWvb

To enter Pbox menus, do the following steps:

1. Enter **`ds`** in the VNMR input window.
2. Click on **Pbox**.

For example, to create a simple excitation pulse, such as an E-BURP-1 shaped pulse, do the following steps:

1. Click on **Pbox** > **90**.
2. Select an excitation region using cursors
3. Click on **e-Burp1** > **Close** > **Name** > **Close**.

The Name button is optional and can be omitted. The shape files are stored under a default name (usually Pbox.RF).

To create a multiply-selective pulse, do the following steps:

1. Click on **Pbox** > **90**.
2. Select an excitation region using cursors.
3. Click on **e-Snob**.
4. Select the next region using cursors.
5. Click on **e-Snob**.
6. Repeat steps 4 and 5 as many times as required.
7. Click on **Close** > **Close**.

To create 4-ply selective excitation pulses for phase encoding (e.g., in experiments using the Hadamard transform), use the following sequence:

1. Click on **Pbox** > **90**.

2. Click on **Options** > **Phase** > **Return**.
3. Select an excitation region using cursors
4. Click on **e-Burp1**.
5. Repeat steps 1 through 4 as required.
6. Click on **Close** > **Name** > **Close**.

To create inversion and refocusing pulses:

1. Click **Pbox** > **180**.
2. Select an inversion band using cursors.
3. Click on **iBurp2** > **Close** > **Bloch**, enter **y**, and click on **Close**.

In the last example, a Bloch simulation is activated and the inversion profile is displayed on top of the spectrum. A relaxation-sensitive simulation on a refocusing pulse can be carried out by using the following menus:

1. Click on **Pbox** > **180** > **Refoc**.
2. Select a refocusing band using cursors.
3. Click on **reBurp** > **Close** > **T1** > **T2** > **Bloch**, enter **y**, and click on **Close**.

If the spectrum is not available, as is frequently the case with homo-decoupling in indirectly detected experiments, the bandwidth and offset can be manually entered, using the Options button. For example, off-resonance SEDUCE-1 decoupling centered on carbonyls can be created as follows:

1. Click on **Pbox** > **Homo-dec** > **Options** > **Offset** > **Bandwidth** > **Return**
2. Click on **Seduce1** > **Close** > **Name** > **Close**.

For homodecoupling in the directly detected dimension, it is necessary to specify the decoupler duty cycle. For adiabatic decoupling, the J-coupling must also be specified:

1. Click on **Pbox** > **Homo-dec** > **Adiabatic** (set J = 10 Hz for H-H decoupling).
2. Select decoupling band using cursors.
3. Click on **WURST-2** > **Close** > **Dutycyc** > **Name** > **Close**.

Heteronuclear decoupling and mixing sequences usually do not need to be shifted off-resonance and the menu sequence can be very simple:

1. Click on **Pbox**.
2. Click on **Mixing** > **bw (Hz)** > **DIPSI-3** > **Name** > **Close**.

A default supercycling is usually assigned to all waveforms in the directories `wavelib/decoupling` and `wavelib/mixing`. However, the supercycle can be changed or assigned to other (nondecoupling) shapes either by using the Phase button in the Options menu or using the **Sucyc** button in the Close menu. It is necessary to click the **Sucyc** button several times in order to create a nested supercycle:

1. Click on **Pbox** > **Het-dec** > **bw (Hz)** > **WALTZ16** > **Name**.
2. Click on **Sucyc**, enter **`t5`**, click on **Sucyc**, enter **`m4`**, and click on **Close**.

It is even simpler to create shaped gradients:

- Click on **Pbox** > **Grad** > **h-Sine**.

The Other button in different menus or the Wavelib button provide access to all `wavelib` items. The Wavelib sequence of menus is slightly different from the usual menus. For example, a multiply selective adiabatic inversion pulse using sech (hyperbolic secant) shape is created as follows:

1. Click on **Pbox** > **Wavelib** > (select **inversion/**) **Ch Dir** > (select **sech**).
2. Click on **Set Shape**.
3. Select inversion region using cursors.
4. Click on **Set Wave**.
5. Repeat step 4 as required.
6. Click on **Close** > **Name** > **Close**.

Information about various shapes is also accessed through the Wavelib menu:

1. Click on **Pbox** > **Wavelib**.
2. Select **decoupling/** and click on **Ch Dir**.
3. Select **MPF7** and click on **i?**
4. Repeat step 3 as required.

Pbox Macro Reference

Although most of needs for generating selective pulses can be satisfied by using Pbox menus, a set of macros is provided for those who prefer macros over menus. The following table lists the macros in the order of decreasing importance. For additional information on Pbox macros, refer to the manual *VNMR Command and Parameter Reference*.

`opx`	Opens `Pbox`, writes the `Pbox.inp` file header, and resets parameters `r1-r7` and `n1-n3.`
`selex`	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also `sets r1` to excitation bandwidth and `r2` to offset. `selex` uses the `pbox_bw` and `putwave` macros.
`cpx`	Calls the `Pbox` command, which generates the specified waveform as defined by the `Pbox.inp` file. `cpx` also checks if parameters `ref_pwr` and `ref_pw90` exist in the current experiment and puts their values into the `Pbox.inp` file. If the parameters do not exist, `cpx` creates them and asks the user for parameter magnitudes.
`setwave`	Sets up a single excitation band in the `Pbox.inp` file. An unlimited number of waves can be combined by reapplying `setwave`.
`putwave`	Sets up a single excitation band in the `Pbox.inp` file. An unlimited number of waves can be combined by reapplying `putwave`.
`pxshape`	Generates a single-band waveform based on wave definition provided as a single string of wave parameters.
`pboxpar`	Adds a parameter definition to the `Pbox.inp` file.
`pboxget`	Extracts calibration data from the file `shapefile.ext` generated by `Pbox` or, if the file name is not provided, from the `pbox.cal` file containing parameters of the last created Pbox shape file. Note that the parameter is not changed by this macro if it was set to `'n'` (not used)!
`pbox_pw`	Extracts pulse length from the file `shapefile.RF` generated by `Pbox` or, if the file name is not provided, from `pbox.cal` file containing parameters of the last created Pbox shape file.

`pbox_pwr`	Extracts the power lever from the file `shapefile.ext` generated by `Pbox` or, if the file name is not provided, from the `pbox.cal` file containing parameters of the last created Pbox shape file. Note that the parameter will not be changed by this macro if previously set to `'n'` (not used).
`pbox_pwrf`	Extracts the fine power lever from the file `shapefile.ext` generated by `Pbox` or, if the file name is not provided, from the `pbox.cal` file containing parameters of the last created Pbox shape file. Note that the parameter will not be changed by this macro if it was previously set to `'n'` (not used).
`pbox_dmf`	Extracts the `dmf` value from the file `shapefile.DEC` created by `Pbox` or, if the file name is not provided, from the `pbox.cal` file containing parameters of the last created Pbox shape file.
`pbox_dres`	Extracts the `dres` value from the file m `shapefile.DEC` created by `Pbox` or, if the file name is not provided, from the `pbox.cal` file containing parameters of the last created Pbox shape file.
`pbox_name`	Extracts name of the last shape file generated by `Pbox` and stored in the `pbox.cal` file. Note, that the file name extension is not stored explicitly and is not provided by this macro.
`dshape`	Displays real (X) and imaginary (Y) components of a shaped pulse. Any type of waveform (.RF, .DEC or .GRD) can be displayed.
`pshape`	Generates a single-band waveform based on wave definition provided as a single string of wave parameters.
`dshapef`	Displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in `pbox.fid` file.
`dshapei`	Interactively displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in `pbox.fid` file
`dprofile`	Displays the X, Y, and Z excitation (inversion) profile for a pulse shape generated by the Pbox software.
`pprofile`	Plots the X, Y, and Z excitation (inversion) profile for a pulse shape that has been generated with the Pbox software. If a shape name is not provided, the last simulation data stored in `shapelib/Pbox.sim` are plotted.
`pph`	Prints out the shape file header (i.e., all lines starting with #).
`pbox_bw`	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also sets `r1` to excitation bandwidth and `r2` to offset. This macro is used mainly in Pbox menus and macros.
`pbox_bws`	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also sets `r1` to excitation bandwidth and `r2` to offset. Note, the left cursor should be placed on the left side of the excitation band and the right cursor on resonance of the solvent signal. This macro is mainly used in Pbox menus and macros.
`pbox_rst`	Resets `r1=0`, `r2=0`, `r3=0`, `r4=0`, `n2='n'`, `n3=''`, and adds some standard comment lines to the `Pbox.inp` file. This macro is used in menus and other Pbox macros.
`pbox_files`	This macro is used only in conjunction with Pbox file menus.

Pbox PSG Statements

Pulse programs can be significantly simplified using a set of Pbox PSG statements. Because the calibration parameters are stored in the shape file header in a predefined form, they can be directly retrieved from the Pbox shape file within the pulse program. Storage reduces the number of statements in the pulse program and also the number of created and displayed parameters in the current experiment, especially in the case of complex multidimensional

pulse sequences. Because parameters like `dres`, `dmf`, and `selpw` are normally not changed, there is no need to create and display them.

The Pbox psg statements are provided as `Pbox_psg.h` `include` file, which is normally stored in the `/vnmr/psg` directory. Compare the two DPFGSE pulse programs in Listing 1 and Listing 2.

Listing 1. Text of DPFGSE Pulse Program, Version A

```
/* dpfgse.c - version A */
#include <standard.h>
#include <Pbox_psg.h>            /* Pbox psg-functions included */
static int ph1[2] = {0,2};
static int ph2[8] = {0,0,2,2,1,1,3,3}
pulsesequence()
{
   double  gzlvl1 = getval("gzlvl1"),
           gzlvl2 = getval("gzlvl2");
/* Declare & retrieve shape parameters */
   shape   grdpat = getGshape("grdpat"),    /* .GRD shape */
           pwpat = getRshape("pwpat"),      /* .RF shape */
           decseq = getDshape("decseq");    /* .DEC shape */
   char   Hdecflg[MAXSTR];

   getstr('Hdecflg', Hdecflg);
           decseq.pwr = getval('decpwr');   /* optional */
   settable(t1, 2, ph1);
   settable(t2, 8, ph2);
/* Pulse sequence starts here */
   status(A);
delay(d1);

   status(B);
obspower(tpwr);
rgpulse(pw, t2, rof1, rof2);           /* hard ninety */

pbox_grad(grdpat, gzlvl1, 0.0, d2);    /* Pbox shaped gradient */
pbox_pulse(pwpat, t1, rof1, rof2);     /* Pbox selective 180 pulse */
pbox_grad(grdpat, gzlvl1, 0.0, d2);    /* Pbox shaped gradient */

pbox_grad(grdpat, gzlvl2, 0.0, d2);
pbox_pulse(pwpat, t1, rof1, rof2);
pbox_grad(grdpat, gzlvl2, 0.0, d2);

   status(C);
setreceiver(t2);
   if (Hdecflg[0] == 'y') /* Pbox homo-decoupling */
homodec(decseq);
}
```

Note that power, fine power, and pulse length of shaped pulses are internally adjusted by the `pbox_pulse` statement making pulse program code compact and easy to read. Fine power is set only if the external attenuation mode (`attn=e` in `Pbox.inp`) was used to generate the shape file. Although, you can still have access to all experimental parameters via the `getval` statements, in the majority of applications, these parameters do not need to be changed. For example, because of the high tolerance of adiabatic pulses to B1-inhomogeneity, possible incorrect calibration of this pulse is not a concern, and the only

Listing 2. Text of DPFGSE Pulse Program, Version B

```
/* dpfgse.c - version B */
#include <standard.h>

static int ph1[2] = {0,2};
static int ph2[8] = {0,0,1,1,2,2,3,3};

pulsesequence()
{
   double   selpwr = getval("selpwr"),
            selpw = getval("selpw"),
            gt1 = getval("gt1"),
            gzlvl1 = getval("gzlvl1"),
            gt2 = getval("gt2"),

   gzlvl2 = getval("gzlvl2"),
            gof1 = 0.05*d2,
            gof2 = 0.95*d2,
            dutyc = getval("dutyc"),
            decpwr = getval("decpwr"),
            decres = getval("decres"),
            decdmf = getval("decdmf"),
            dpls90 = 1.0/decdmf,
            decdel = dutyc/sw,
            acqdel = (1.0-dutyc)/sw;

   char     decflg[MAXSTR], decseq[MAXSTR];

   getstr("pwpat", pwpat);
   getstr("decflg", decflg);
   getstr("decseq", decseq);
   getstr("satmode", decseq);
   satpwr = getval("satpwr");
   satdly = getval("satdly");

   if ((dutyc > 0.2) && (decpwr > 49.0))
   {
      printf("decpwr too high! Aborting...\n");
      abort(1);
   }

   if( satpwr > 10.0)
   {
      printf("satpwr too high! Aborting...\n");
      abort(1);
   }

   settable(t1, 8, rec);
   settable(t2, 2, phb);

      status(A);
   if (satmode[0] == 'y')
   {
   obspower(satpwr);
   rgpulse(satdly, zero, rof1, rof2);
   delay(d1-satdly);
```

Listing 2. Text of DPFGSE Pulse Program, Version B (continued)

```
   }
   else
   {
      delay(d1);
   }

      status(B);
   obspower(tpwr);
   rgpulse(pw, t1, rof1, rof2);   /* hard 90 */
   obspower(selpwr);

   delay(gof1);
   rgradient('z',gzlvl1);
   delay(gt1);
   rgradient('z',0.0);
   delay(gof2);

   shaped_pulse(pwpat, selpw, t2, rof1, rof2);
   delay(gof1);
   rgradient('z',gzlvl1);
   delay(gt1);
   rgradient('z',0.0);
   delay(gof2);

   delay(POWER_DELAY);
   delay(gof1);
   rgradient('z',gzlvl2);
   delay(gt2);
   rgradient('z',0.0);
   delay(gof2);

   shaped_pulse(pwpat, selpw, t2, rof1, rof2);
   delay(gof1);
   rgradient('z',gzlvl2);
   delay(gt2);
   rgradient('z',0.0);
   delay(gof2);

      status(C);
   setreceiver(t1);
   if (decflg[0] == 'y')/* homo-decoupling */
   {
      delay(alfa + 1/(1.3*fb) - PRG_START_DELAY - PRG_STOP_DELAY);
      txphase(zero);
      obsprgon(decseq, dpls90, decres);
      initval(np/2.0, v14);
      starthardloop(v14);
         acquire(2.0, acqdel);      /* explicit acquisition */
         rcvroff(); xmtron();
         delay(decdel); xmtroff(); rcvron();
      endhardloop();
      obsprgoff();
   }
}
```

parameter requiring adjustment in the previous example is power level for homonuclear decoupling.

Pbox PSG statements can also be used to generate the shaped gradient:

```
pbox_grad(pattern, amp, gof1, gof2)
```

where `gof1` and `gof2` are (recovery) delays. The gradient pulse width is retrieved from the .GRD file shape header (defaulted to 1 ms by Pbox).

Shaped gradients for spectrometers with no PFG waveform generators is another feature available with the `Pbox_psg.h include` file, and the syntax is almost the same (except loops is substituted by number of steps, `np`):

```
shapedgradient(pattern, width, amp, channel, np, wait);
```

The `shapedgradient` macro in the `/vnmr/psg/macros.h` file needs to be redefined to implement shaped gradients on spectrometers with no gradient WFG as follows:

```
#define
shapedgradient(pulsefile,pulsewidth,gamp0,which,loops,  \
   wait_4_me)shaped_gradient((int)(loops),  \
   (double)(gamp0),(double)(pulsewidth),which)
```

Standard Pbox PSG Statements

`shape` **Declares shape parameters in VNMR pulse program**

Description: Declares shape parameters in VNMR pulse program.

Examples:
```
shape grdpat, pwpat, decpat;
```

`getRshape` **Retrieves shape parameters from .RF shape file header**

Syntax:
```
getRshape("parname");
```

Description: Retrieves shape name, pulse length, power and fine power from the `parname.RF` shape file header provided that `parname` exists in the current experiment. Note that shape parameters can be altered if necessary.

Examples:
```
(shape) pwpat=getRshape("pwpat");
pwpat.pwr=getval("selpwr");       /* optional */
```

`getDshape` **Retrieves shape parameters from .DEC shape file header)**

Syntax:
```
getDshape("parname");
```

Description: Retrieves shape name, pulse length, `dres`, `dmf`, power and fine power from the `parname.DEC` shape file header provided that `parname` exists in the current experiment. Note that shape parameters can be altered if necessary.

Examples:
```
(shape) decpat=getDshape(decpat);
decpat.dmf=getval(dmf2);       /* optional */
```

`getGshape` **Retrieves shape parameters from .GRD shape file header**

Syntax:
```
getRshape("parname");
```

Description: Retrieves shape name and pulse length from the `parname.GRD` shape file header provided that `parname` exists in the current experiment. Note that shape parameters can be altered if necessary.

Examples:
```
(shape) grdpat=getGshape("grdpat");
grdpat.pwr=getval("gt1");       /* optional */
```

getRsh **Retrieves shape parameters directly from .RF shape file header**

Syntax:
```
getRsh('shname');
```

Description: Retrieves pulse length, power and fine power directly from the `shname.RF` shape file header.

Examples:
```
(shape) pwpat=getRsh('esnob_12k');
```

getDsh **Retrieves shape parameters directly from .DEC shape file header**

Syntax:
```
getDsh('shname');
```

Description: Retrieves pulse length, `dres`, `dmf`, power and fine power directly from `shname.DEC` shape file header.

Examples:
```
(shape) decpat=getDsh('COdec');
```

getGsh **Retrieves shape parameters from .GRD shape file header**

Syntax:
```
getGsh('shname');
```

Description: Retrieves shape pulse length directly from the `shapename.GRD` shape file header.

Examples:
```
(shape) grdpat=getGsh('waterg');
```

pbox_pulse **Sets power, performs a shaped pulse**

Syntax:
```
pbox_pulse(shpat,v1,rof1,rof2);
shape   shpat
codeint v1;   /* phase */
double  rof1, rof2;
```

Description: The pulse length and power are internally set, within the Pbox PSG statements. Note, that power is not automatically reset to the previous magnitude.

Examples:
```
pbox_pulse(shpat,v9,rof1,rof1);
pbox_pulse(getRshape('pwpat'),oph,rof1,rof2);
pbox_pulse(getRsh('water_snob'),zero,rof1,rof1);
```

pbox_decpulse **Sets power, performs a shaped pulse on first decoupler**

Syntax:
```
pbox_decpulse(decsh,v1,rof1,rof2);
shape   decsh;
codeint v1;   /* phase */
double   rof1, rof2;
```

Description: Sets power and performs a shaped pulse on the first decoupler channel. The pulse length and power level is internally set.

Examples:
```
pbox_decpulse(decsh,v5,rof1,rof1);
```

pbox_dec2pulse **Sets power, performs a shaped pulse on second decoupler**

Syntax:
```
pbox_dec2pulse(dec2pat,v1,rof1,rof2);
shape   dec2pat;
```

```
codeint v1;        /* phase */
double  rof1, rof2;
```

Description: The pulse length and power are internally set, within the Pbox statements. Note, that decoupler power is not automatically reset to the previous magnitude.

Examples:
```
pbox_dec2pulse(dec2sh,zero,rof1,rof2);
```

pbox_dec3pulse Sets power, performs a shaped pulse on third decoupler

Syntax:
```
pbox_dec3pulse(dec3pat,vi,vj,rof1,rof2);
shape   dec3pat;
codeint vi;        /*phase*/
double  rof1, rof2;
```

Description: The pulse length and power are internally set, within the Pbox PSG statements. Note, that decoupler power is not automatically reset to the previous magnitude.

Examples:
```
pbox_dec3pulse(dec3sh,v2,rof1,rof2);
```

pbox_simpulse Sets power, performs simultaneous shaped pulse

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_simpulse(shpat,decpat,vi,vj,rof1,rof2)
shape   shpat, decpat;
codeint vi, vj;    /* phases */
double  rof1, rof2;
```

Description: Sets power and performs a simultaneous shaped pulse on the transmitter and the first decoupler channels. The pulse lengths and power levels are internally set within the Pbox statement. Note, that both transmitter and decoupler power levels are not automatically reset to the previous magnitude.

Examples:
```
pbox_simpulse(shpat, decpat, zero, v4, rof1, rof2);
```

pbox_sim3pulseSets power, performs a simultaneous shaped pulse

Syntax:
```
pbox_sim3pulse(pwpat,decpat,dec2pat,vi,vj,vk,  \
   rof1,rof2);
shape   pwpat,decpat,dec2pat;
codeint vi,vj,vk;     /* phases */
double  rof1,rof2;
```

Description: Sets power and performs a simultaneous shaped pulse on the transmitter, the first and the second decoupler channels. The pulse lengths and power levels are internally set, within the Pbox statements. Note, that both transmitter and decoupler power levels are not automatically reset to the previous magnitudes.

Examples:
```
pbox_sim3pulse(pwpat,decpat,dec2pat,zero,v5,three, \
rof1,rof2);
```

pbox_sim4pulseSets power, performs a simultaneous shaped pulse

Syntax:
```
pbox_sim4pulse(pwpat,decpat,dec2pat,dec3pat,  \
   vi,vj,vk,vn,rof1,rof2);
shape   pwpat,decpat,dec2pat,dec3pat
codeint vi,vj,vk,vn;   /* phases */
double   rof1,rof2;
```

Description: Sets power and performs a simultaneous shaped pulse on four RF channels. The pulse lengths and power levels are internally set, within the Pbox statements. Note, that both transmitter and decoupler power levels are not automatically reset to the previous magnitudes.

Examples:
```
pbox_sim4pulse(shpat,decpat,dec2pat,dec3pat,
\zero,two,v2,v9,rof1,rof2);
```

pbox_xmtron — Sets power, dmf, and dres, initiates a programmable rf irradiation

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_xmtron(mixpat);
shape        mixpat
```

Description: Sets power, `dmf`, and `dres` and initiates a programmable rf irradiation via the observe transmitter channel. The gating of the observe transmitter (xmtron) is automatically executed within the `pbox_xmtron` statement.

Examples:
```
pbox_xmtron(mixpat); pbox_xmtron(getDshape(decpat));
```

pbox_xmtroff — Terminates a programmable rf irradiation

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_xmtroff();
```

Description: Terminates a programmable rf irradiation via the observe transmitter channel. Note that the power level is not automatically reset to its previous magnitude.

pbox_decon — Sets power, dmf and dres, initiates a programmable rf irradiation

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_decon(decsh);
shape       decsh
```

Description: Sets power, `dmf`, and `dres` and initiates a programmable rf irradiation via the first decoupler channel. The gating of the first decoupler [`decon()`] is internally executed within the `pbox_decon` statement.

Examples:
```
pbox_decon(decsh);
pbox_decon(getDshape(dseq));
pbox_decon(getDsh("WALTZ16"));
```

pbox_decoff — Terminates a programmable rf irradiation

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_decoff();
```

Description: Terminates a programmable rf irradiation via the first decoupler channel. Note that the power level is not automatically reset to the previous value.

pbox_dec2on — Sets power, dmf and dres, initiates programmable rf irradiation

Syntax:
```
pbox_dec2on(dec2sh);
shape       dec2sh;
```

Description: Sets power, dmf and dres and initiates a programmable rf irradiation via the second decoupler channel. Gating of the second decoupler [`dec2on()`] is internally executed within the `pbox_dec2on` statement.

Examples:
```
pbox_dec2on(dec2sh);
pbox_dec2on(getDshape(dseq2));
pbox_dec2on(getDsh(garp1));
```

pbox_dec2off Terminates a programmable rf irradiation

Syntax:
```
pbox_dec2off();
```

Description: Terminates a programmable rf irradiation via the second decoupler channel. Note that the power level is not automatically reset to the previous value.

pbox_dec3on Sets power, dmf, and dres, initiates a programmable rf irradiation

Syntax:
```
pbox_dec3on(dec3sh);
shape       dec3sh;
```

Description: Sets power, `dmf`, and `dres`, and initiates a programmable rf irradiation via the third decoupler channel. Gating of the third decoupler [`dec3on()`] is internally executed within the `pbox_dec3on` statement.

Examples:
```
pbox_dec3on(dec3sh);
pbox_dec3on(getDshape(dseq3));
pbox_dec3on(getDsh("WALTZ16"));
```

pbox_dec3off Terminates a programmable rf irradiation

Syntax:
```
pbox_dec3off();
```

Description: Terminates a programmable rf irradiation via the third decoupler channel. Note that the power level is not automatically reset to the previous value.

pbox_spinlock Sets power and phase, executes a programmable spin lock

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_spinlock(mixshape,mixtime,mixphase);
shape     mixshape;
double    mixtime;
codeint   mixphase;
```

Description: Sets power, fine power, `dmf`, `dres`, calculates the required number of loops and executes a programmable spin lock via the observe transmitter channel.

Examples:
```
pbox_spinlock   (mixsh,mix,zero);
pbox_spinlock(getDshape(mixpat),mix,zero);
pbox_spinlock(getDsh(dipsi2),mix,v2);
```

pbox_decspinlock Sets power and phase, executes a programmable spin lock

Applicability: Not applicable on *MERCURY*.

Syntax:
```
pbox_decspinlock(mixshape,mixtime,mixphase);
shape      mixshape;
double     mixtime;
codeint     mixphase;
```

Description: Sets power, fine power, `dmf`, `dres`, calculates the required number of loops and executes a programmable spin lock via the first decoupler channel.

Examples:
```
pbox_decspinlock(mixsh,mix,zero);
pbox_decspinlock(getDshape(mixpat),mix,zero);
pbox_decspinlock(getDsh(flopsy8),mix,v2);
```

pbox_dec2spinlock Sets power and phase, executes a programmable spin lock

Syntax:
```
pbox_dec2spinlock(mixshape,mixtime,mixphase);
shape     mixshape;
double    mixtime;
codeint   mixphase;
```

Description: Sets power, fine power, `dmf`, `dres`, calculates the required number of loops and executes a programmable spin lock via the second decoupler channel.

Examples:
```
pbox_dec2spinlock(mixsh,mix,zero);
pbox_dec2spinlock(getDshape(mixpat),mix,zero);
pbox_dec2spinlock(getDsh(dipsi3),mix,v2);
```

pbox_dec3spinlock Sets power and phase, executes a programmable spin lock

Syntax:
```
pbox_dec3spinlock(mixshape,mixtime,mixphase);
shape     mixshape;
double    mixtime;
codeint   mixphase;
```

Description: Sets power, fine power, `dmf`, `dres`, calculates the required number of loops and executes a programmable spin lock via the third decoupler channel.

Examples:
```
pbox_dec3spinlock(mixsh,mix,zero);
pbox_dec3spinlock(getDshape(mixpat),mix,zero);
pbox_dec3spinlock(getDsh(clnmlev),mix,v2);
```

pbox_grad Sets gradient length and performs shaped z gradient pulse

Applicability: Systems with no WFG on PFG module (see page 243). On *MERCURY*, installation is not required.

Syntax:
```
pbox_grad(grdpat,gzlvl,gof1,gof2);
shape grdpat;
double gzlvl, gof1, gof2;
```

Description: Sets the gradient length and performs a shaped `'z'` gradient pulse. Unlike the standard `shapedgradient` statement, the gradient is turned off automatically. `gof1` and `gof2` are pregradient and postgradient (recovery) delays.

pbox_xgrad Sets the gradient length and performs shaped x gradient pulse

Applicability: Systems with no WFG on PFG module (see page 243). Not applicable on *MERCURY*.

Syntax:
```
pbox_xgrad(grdpat,gw,gxlvl,gof1,gof2);
shape   grdpat
double   gxlvl, gof1, gof2 gw;
```

Description: Performs a shaped `'x'` gradient pulse of length `gw`. Unlike the standard `shapedgradient` statement, the gradient is automatically turned off. `gof1` and `gof2` are pregradient and postgradient (recovery) delays.

pbox_ygrad **Sets the gradient length and performs shaped y gradient pulse**

Applicability: Systems with no WFG on PFG module (see page 243). Not applicable on *MERCURY*.

Syntax:
```
pbox_ygrad(grdpat,gw,gylvl,gof1,gof2);
shape      grdpat;
double     gylvl,gof1,gof2, gw;
```

Description: Performs a shaped `'y'` gradient pulse of length `gw`. Unlike the standard `shapedgradient` statement, the gradient is automatically turned off. `gof1` and `gof2` are pregradient and postgradient (recovery) delays.

pbox_zgrad **Sets the grad length and performs a shaped z grad pulse**

Applicability: Systems with no WFG on PFG module (see page 243). On *MERCURY*, installation is not required.

Syntax:
```
pbox_zgrad(grdpat,gw,gzlvl,gof1,gof2);
shape      grdpat
double     gzlvl,gof1,gof2
```

Description: Performs a shaped `'z'` gradient pulse of length `gw`. Unlike the standard `shapedgradient` statement, the gradient is automatically turned off. `gof1` and `gof2` are pregradient and postgradient (recovery) delays.

Miscellaneous PSG Statements

homodec **Set power, dutyc, dmf, and dres, then executes irradiation**

Applicability: Not applicable on *MERCURY*.

Syntax:
```
homodec(decseq)
shape decseq
```

Description: Sets power, `dmf`, and `dres`, and executes a programmable rf irradiation via the observe transmitter during the acquisition in a time-shared mode.

Examples:
```
homodec(Hdecsh);
homodec(getDshape(Hdecsh));
homodec(getDsh(Hhwurst));
```

pfg_pulse **Perform a rectangular z gradient pulse**

Syntax:
```
pfg_pulse(gzlvl,gw,gof1,gof2);
double gzlvl, gw, gof1, gof2
```

Description: `gw` is gradient pulse width, `gof1` and `gof2` are pregradient and postgradient (recovery) delays.

Examples:
```
pfg_pulse(gz2lvl,gt2,rof1,grec);
```

presat **Performs a long rf pulse (presaturtion)**

Syntax:
```
presat();
```

Description: Performs a long rf pulse (presaturtion) via the observe transmitter. Uses the standard `presat` parameters `satpwr`, `satdly`, and `d1`. `presat()` is executed only if `satdly` is greater than 0.0 (`satflg` and `satfrq` are not active).

Examples:
```
presat();
```

pre_sat **Performs a long rf pulse (presaturtion)**

Syntax: `pre_sat();`

Description: Performs a long rf pulse (presaturtion) via the observe transmitter. It uses the standard `presat` parameters `satmode` and `satfrq` in addition to `satpwr`, `satdly`, and `d1`.

Examples: `pre_sat();`

setlimit **Sets a (safety) limit for the given parameter**

Syntax:
```
setlimit(name, parameter, limit);
char name[MAXSTR];
double parameter, limit;
```

Description: Sets a (safety) limit for the given parameter. The execution of the pulse program is terminated if the parameter magnitude exceeds the given (safety) limit.

Examples: `setlimit('dpwr2', dpwr2, 49);`

Pulse Shaping “On-Fly”

It is often convenient to create pulse shapes from within a pulse program (on-fly), i.e. during acquisition (`go`), which enables you to keep the experiment setup very simple and independent of spectrometer frequency. In “C,” pulse shaping is implemented using the `system()`statement. For example:

```
system("Pbox sh.RF -w \"esnob 20p 170p\" -attn e -refofs 55p
         -sfrq 150.05 -ref_pwr 51 -ref_pw90 60.2");
```

A more convenient and flexible approach is to construct the command line first and then execute it using the `system()` statement. This method enables you to retrieve the necessary parameters from the experiment. The following example shows how to create and use a wide-band adiabatic inversion pulse with the maximum efficiency:

```
...
char cmd[MAXSTR];
double pwxlvl, pwx;
...
if((getval("arraydim") < 1.5) || (ix==1)) /* execute only once */
{
sprintf(cmd, "Pbox ad180.RF -w \"cawurst-10 %.1f/%.6f\" -s
1.0 -0",1.0/pwx, 20.0*pwx);
system(cmd);
}
...
      decpower(pwxlvl);
      decshaped_pulse("ad180", 20.0*pwx, zero, rof1, rof1);
...
```

Pbox_psg.h include Pulse Sequence Statements

For even more convenience and readability, the following pulse sequence statements are provided in the `Pbox_psg.h include` file.

opx **Initiates Pbox within pulse program**

Syntax:
```
opx(shname);
char shname[MAXSTR];
```

Description: Initiates Pbox from within a pulse program. Similar to the `opx` macro, except the `Pbox.inp` file is not created.

Examples:
```
opx("water");
```

setwave — Writes wave string into Pbox buffer

Syntax:
```
setwave(wvstr);
char wvstr[MAXSTR];
```

Description: Writes a wave definition string into a Pbox buffer. Used for pulse shaping from within a pulse program (on fly). Similar to the `setwave` macro, except the `Pbox.inp` file is not created.

Examples:
```
setwave("eburp1 200.0 -1.2k");
setwave("esnob 20p 170p");
```

putwave — Writes wave string into Pbox buffer

Syntax:
```
putwave(sh,bw,ofs,st,pha,fla);
char      sh[MAXSTR];
double    bw,ofs,st,pha,fla;
```

Description: Writes a wave definition string into a Pbox buffer. Used for pulse shaping from within a pulse program (on fly). Similar to the `putwave` macro, except the `Pbox.inp` file is not created. Unlike the macro, a full set of arguments is required. Zero can be used to request a default value.

Examples:
```
putwave("eburp1",200.0,-1200.0,1.0,90.0,0.0);
```

pboxpar — Writes a Pbox parameter into Pbox buffer

Syntax:
```
pboxpar(parname, parval);
char    parname[MAXSTR];
double  parval;
```

Description: Writes a Pbox parameter into a Pbox buffer. Used for pulse shaping from within a pulse program (on fly). Note that `parval` is a real number.

Examples:
```
pboxpar("stepsize",2.0);
pboxpar("sfrq",dfrq);
```

pbox_par — Writes a Pbox parameter into Pbox buffer

Syntax:
```
pboxpar(parname,parval);
char    parname[MAXSTR],parval[MAXSTR];
```

Description: Writes a character type Pbox parameter into a Pbox buffer. Used for pulse shaping from within a pulse program (on fly). Note that `parval` is a string variable.

Examples:
```
pboxpar("attn","e");
pboxpar("sucyc","t5,m4");
pboxpar("refofs","55p");
```

cpx — Executes Pbox within pulse program

Syntax:
```
cpx(ref_pw90,ref_pwr);
double ref_pwr,ref_pw90;
```

Description: Executes Pbox from within a pulse program. Similar to the `cpx` macro.

Examples:
```
cpx(ref_pw90,ref_pwr);
cpx(compH*pw90,tpwr);
```

pbox_get **Retrieves shape parameters within pulse program**

Syntax: `pbox_get();`

Description: Retrieves shape parameters from the `pbox.cal` file and sets `pbox_pw`, `pbox_pwr`, `pbox_pwrf`, `pbox_dres` and `pbox_dmf` from within a pulse program. These parameters can then be assigned to appropriate variables.

Examples:
```
pbox_get();
selpw=pbox_pw;
selpwr=pbox_pwr;
```

isarry **Checks whether given parameter is arrayed**

Syntax:
```
isarry(parname);
char parname[MAXSTR];
```

Description: Returns 1 if parameter is arrayed and zero otherwise.

Examples: `isarry("selpwr");`

shonfly.c Sequence

The following two examples show a simple shonfly.c sequence. You must check the `arraydim` and `ix` parameters to avoid excessive pulse shaping in arrayed experiments.

Version A

```
/* shonfly.c - version A, allows to array ofs */
#include <standard.h>
#include <Pbox_psg.h>

static double selpw=0.0, selpwr=0.0;
static char   sharr[MAXSTR];

pulsesequence()
{
   double    n=0.0,
             ofs = getval("ofs"),     /* frequency offset */
             ref_pwr = getval("ref_pwr"),
             ref_pw90 = getval("ref_pw90");
   char      repflg[MAXSTR];

   getstr("pwpat", pwpat);       /* Retrieve the variables */
   getstr("repflg", repflg);

/* create a shaped pulse using Pbox macros only when needed */

 if((getval('arraydim') < 1.5) || (ix==1) || isarry("ofs"))
{
     sprintf(sharr, "%s_%ld", pwpat, ix);
     opx(sharr);                        /* open Pbox */
     putwave("qsneeze", 200.0, ofs, n, n, n);
     pboxpar("stepsize", 10.0);          /* stepsize in us */
     if (repflg[A] == 'y')  pbox_par("reps", "2");
     else                  pbox_par("reps", "0"); /* silent mode */
```

```
     cpx(ref_pw90, ref_pwr);                      /* close Pbox */
     pbox_get();                    /* retrieve shape data */
     selpwr = pbox_pwr;                 /* use Pbox power */
     selpw  = pbox_pw;                  /* use Pbox pw    */
}

/* THE PULSE PROGRAM STARTS HERE */

status(A);
  obspower(selpwr);
  delay(d1);
status(B);
  shaped_pulse(sharr, selpw, oph, rof1, rof2);
status(C);
}
```

Version B

```
/* shonfly.c - version B, allows to array ofs */
#include <standard.h>
#include <Pbox_psg.h>

static char   sharr[MAXSTR];

pulsesequence()
{
double  reps=0.0,                       /* silent mode */
         ofs = getval("ofs"),           /* frequency offset */
        ref_pwr = getval("ref_pwr"),
        ref_pw90 = getval("ref_pw90");
char    repflg[MAXSTR], cmd[MAXSTR];

getstr("repflg", repflg);        /* Retrieve the variables */
if (repflg[A] == 'y') reps = 2.0;

/* create a shaped pulse using Pbox macros only when needed */

if((getval("arraydim") < 1.5) || (ix==1) || isarry("ofs"))
{
   sprintf(sharr, "shtst_%ld", ix);
   sprintf(cmd, "Pbox %s -w \"qsneeze 200.0 %.2f\" -s 10",
                sharr, ofs);
   sprintf(cmd, "%s -p %.0f -l %.2f %.0f", cmd, ref_pwr,
                 ref_pw90*1.0e6, -reps);
   system(cmd);
}

/* THE PULSE PROGRAM STARTS HERE */

status(A);
  delay(d1);
status(B);
  pbox_pulse(getRsh(sharr), oph, rof1, rof2);
status(C);
}
```

Although version B is less readable, it is clearly more compact and efficient.

Pbox UNIX Commands

The `Pbox` program is always executed when a shaped pulse is created. Any of the Pbox parameters can be used as an argument followed by the parameter value. The arguments and shortcuts listed in Table 43 are available. Note that the output filename is optional and is always the first argument. .

Table 43. Pbox Commands and Parameters (continued)

Command	*Parameter*	*Action*
`Pbox*`	`-b time`	Activate Bloch simulator, `opt=a` (add), `s` (subtract), or time in sec.
	`-c`	Calibrate only, do not create a shape file.
	`-f file`	Set name of the output file.
	`-h wave`	Print wave file header.
	`-i wave`	Print wave file parameters.
	`-l ref_pw90`	Length (in µs) of reference pw90 pulse.
	`-o`	List options.
	`-p ref_pwr`	Reference power level (dB).
	`-r file`	Reshape Pbox pulse.
	`-s stepsize`	Define the length (in µs) of a single step in the waveform.
	`-t wave`	Print shape title from wave file.
	`-u userdir`	Set user home directory.
	`-w wavestr`	Set wave definition string.
	`-v`	Run in verbose mode. Also print Pbox version.
	`-x`	Prints all Pbox parameters.
	`-value`	Sets reps to value.
`Pxsim`		Used in Pbox menus and macros for simulation of excitation profiles of shaped pulses.
`Pxfid`		Used by `dshape` and `dshapei` to format shape file into a FID-format text file.
`Pxspy`		Converts alien shapes (.RF, .DEC and .GRD) into Pbox compatible file format. Essentially converts a time-domain shape file into (frequency-domain) Fourier coefficients, which can be used to create a wave file in the `wavelib` directory.

Examples:

```
Pbox -i eburp2
Pbox newshape -wc 'eburp1 450 -1280.0' -1
Pbox sel.RF -w 'eburp1 420 -800' 'eburp1 420 1200'
Pbox -w 'eburp1 200 -1200' -attn e -pl 45 54.2 -b
Pbox tst.RF -w 'esnob 20p 170p' -sfrq 150.02 -refofs 55p -refpwr 45  \
-ref_pw90 54.2
```

Chapter 8. Variable Temperature Operation

Sections in this chapter:

- 8.1 “Startup,” this page
- 8.2 “Operating Procedures,” page 260
- 8.3 “Temperature-Related Command,” page 262
- 8.4 “Operating Recommendations,” page 263
- 8.5 “VT Controller Safety Circuits,” page 264
- 8.6 “VT Interlock Parameters,” page 265

This chapter describes startup and operation of the optional variable temperature (VT) unit. A VT unit is available for all Varian NMR spectrometers to vary the temperature of the sample.Table 44 lists commands and parameters associated with VT operation.

Table 44. Variable Temperature Unit Operation Commands and Parameters

Command	
`acqmeter<('host')>`	Open the Acqmeter window
`dgs`	Display group shims, automation parameters
`ga<('nocheck')>`	Submit experiment to acquisition and FT result
`go<(<'acqi><,'nocheck'><,'nosafe'>)>`	Submit experiment to acquisition
`sethw('vt','reset')`	Reset VT controller (not on *GEMINI 2000*)
`su`	Submit a setup experiment to acquisition
`tempcal(solvent)<:temperature>`	Temperature calculation
Parameter	
`in` {'n','w','y'}	Lock and spin interlock
`pad`*	Preacquisition delay
`temp` {–150 to +2000, in deg. C}	Sample temperature
`tin` {'n','w','y'}	Temperature interlock
`vtc` {0 to 50, in deg. C}	Variable temperature cutoff point
`vttype` {2,0}	Variable temperature controller type
`vtwait` {number, in sec}	Variable temperature wait time
* pad {0 to 4095 (*GEMINI 2000*), 0 to 8190 (others), in seconds}	

8.1 Startup

On systems equipped with the VT unit, the parameter `temp`, set by the user, changes the internal probe temperature. A thermocouple senses the temperature, which the VT controller continuously displays on the front panel. The controller compares the user-requested value with the current probe temperature and changes the heater current accordingly. The VT controller then reports the temperature of the gas flow and status to the spectrometer through a serial port at the rear of the console. The `vtc` parameter (for

"variable temperature cutoff"), also set by the user, determines the temperature below which the gas is cooled. The values of the `temp` and `vtc` parameters are shown in the "SPECIAL" section of the parameter display called by the `dgs` macro.

Starting up the VT unit takes the following steps:

1. Refer to the installation manual to make sure that the hardware is installed and connected properly, and that the VT controller is calibrated correctly.
2. If the system power has been off or the VT unit has been disconnected from the probe, reset the VT controller by pressing the POWER switch to turn the unit off, then pressing POWER again to turn it on. The VT controller also can be reset from the Temperature Control window (see page 262) or by entering the VNMR command `sethw('vt','reset')`.

CAUTION: **For VT and probe operation, use either dry nitrogen gas or air. A mixture of nitrogen gas and air can cause spikes in the baseline adjacent the large peaks in the spectra. For temperatures above 100°C, the use of air as the VT gas is not recommended. Such use will destructively oxidize the heater element and the thermocouple.**

3. Use dry nitrogen gas if the requested temperature is over 100°C or below the dew point or 0° C, whichever is higher. Otherwise, air may be used as the VT gas. If the requested temperature is below –40°C, dry nitrogen gas is recommended for cooling the bearing, spinner, and decoupler. This prevents moisture condensation in the probe and spinner housing.

   The source of heating or cooling gas is not automatically selected. To use nitrogen, you must attach a nitrogen gas source to the VT system. The same is true when using air. The VT system only selects the routing of the gas flow.
4. Use the flow control meter on the magnet leg to adjust the flow to about 10 LPM (as shown on the flow gauge).
5. A sample that can be handled at ambient temperature can now be placed in the probe, NMR lock obtained, and field homogeneity adjusted. Samples that cannot be handled at ambient temperature should wait until the system reaches the requested temperature.

8.2 Operating Procedures

The following procedures are recommended:

1. (Highland VT units) The power switch is located on the back panel. The heater on/off switch is located on the front panel.

   (Oxford VT units) Although the VT unit should be left on at all times, it's a good idea to check that the VT controller power is on (front panel button pressed in with the light on).

   If the VT controller is off and you cannot turn it on, run the `config` program and check that the VT Controller label is set to Present.
2. Enter the acquisition parameters for the experiment as usual.
3. Enter **`acqmeter`** to open the Acqmeter display. Click in the display and select VT in the popup menu. To change the look of the display, select Properties from the popup menu.

4. Enter the desired temperature value(s) using the `temp` parameter.
5. If the `temp` parameter is an array, set the **`pad`** parameter for preacquisition delay that allows sufficient time for the sample to equilibrate after a temperature change. The system will then wait `pad` seconds in between each temperature before starting data acquisition. Delays of several minutes are optimum because the sample will take longer to equilibrate than it takes the VT controller to stabilize the heating/ cooling gas at the set point.
6. Set the **`vtc`** parameter to a temperature near the ambient VT gas temperature (normally `vtc` is correct and need not be changed). Based upon the value of `vtc` compared to the value of `temp`, the system will route the VT gas flow for either heating or cooling.
7. Start temperature control by entering a **`su`**, **`go`**, or **`ga`** command. These commands act as follows:
   - If `su` is entered, the temperature control and acquisition hardware controls are set and the sample temperature changed to the desired temperature. The experiment will not be started when the desired temperature is reached. After waiting `pad` seconds, `go` or `ga` must be issued before data acquisition will begin.
   - If a `go` or `ga` is entered, the same actions as `su` occur, except that after reaching the desired temperature, the system waits `pad` seconds then begins the pulse sequence and data acquisition. The `pad` time delay will occur every time the temperature is changed under program control.

CAUTION: **Do not use aromatic, ketone (including acetone), and chlorinated solvents in the coolant bucket. Such coolant media attack the standard polystyrene bucket. Another type of container must be used (not supplied by Varian).**

After entering `su`, `go`, or `ga`, the selection of the VT gas routing occurs, and the VT controller begins to control the gas temperature in the probe at the requested value of `temp`. The temperature readout will begin to change and the VT indicator light will begin flashing. At this time, if the requested temperature is below ambient, add coolant liquid to the coolant bucket.

CAUTION: **Operating the system with the coolant bucket filled with liquid nitrogen and with the value of `temp` greater than the value of `vtc` results in the condensation of liquid nitrogen inside the exchanger coil tube. If the exchanger coil is then warmed above –210°C or if nitrogen gas is passed through the coil (when `temp` is less than `vtc`), very cold liquid nitrogen is forced through the transfer line and into the probe. This will cause a sudden pressure surge in the transfer lines and probe as the liquid nitrogen boils, and it can blow the flexible connector apart. If the liquid nitrogen reaches the glass components of the probe and sample tube, the glass will probably break. Instrument damage can be avoided by following these precautions:**

Do not immerse the exchanger coil in liquid nitrogen when no nitrogen gas is flowing through the coil.

Do not stop the VT nitrogen gas flow while the exchanger is immersed in liquid nitrogen.

Arrayed VT experiments that have a temperature range from above *vtc* to below *vtc* should be set up starting at the lowest temperature and ending at the highest temperature. When the experiment passes the *vtc* crossover, remove the liquid nitrogen coolant.

To avoid water in the exchanger when the low temperature experiment is complete, warm up the exchanger by removing it from the liquid nitrogen and maintain a flow of dry nitrogen until room temperature is reached.

When the temperature reaches the value requested by `temp` (it may initially overshoot), the VT indicator light stays on steadily. A sample that could not be handled at ambient temperature can now be transferred into the probe. The VT readout is the temperature of the cooling/heating gas and may be different from the true sample temperature. The exact temperature of the sample is correctly determined by a calibration curve that must be constructed for each probe, and must include flow rate and equilibration time. Refer to the VT installation manual for the NMR calibration method.

CAUTION: **Before running sealed samples at elevated temperatures, check the samples in an oven at a temperature higher than the highest temperature during the experiment. When heated, volatile materials in a sealed tube can build up high pressures. If the tube ruptures while in the probe, the glass components and insert coil will probably be destroyed. For the same reason, do not insert into the probe any sealed sample of volatile material that must be kept cold to avoid excessive pressure buildup. Be sure the probe has equilibrated to a safe, cold temperature before inserting the sample.**

8.3 Temperature-Related Command

For systems equipped with the optional variable temperature controller, the `temp` command opens the Temperature Control window, shown in Figure 61.

The Temperature Control window can be used for the following purposes

- Turn off temperature control.
- Set temperature control on at a specified temperature in degrees C.
- Enable temperature control from within an experiment using the `temp` parameter and the `su`, `go`, `ga`, or `au` commands and macros.
- Alternatively, turn off experiment control of the temperature and allow only the Temperature Control window (and the command `sethw`) to set the temperature.
- Reset the temperature controller when the temperature cable is reconnected to a probe.

If the temperature is controlled only through the Temperature Control window, two actions (to be taken after a temperature error) can be selected:

- Display a warning but continue acquisition.
- Stop acquisition and display a warning.

If experiment control of temperature is selected, the two previous selections appear faded because they are inoperative, and the selection of the action to be taken after a temperature error is provided by the parameter `tin`.

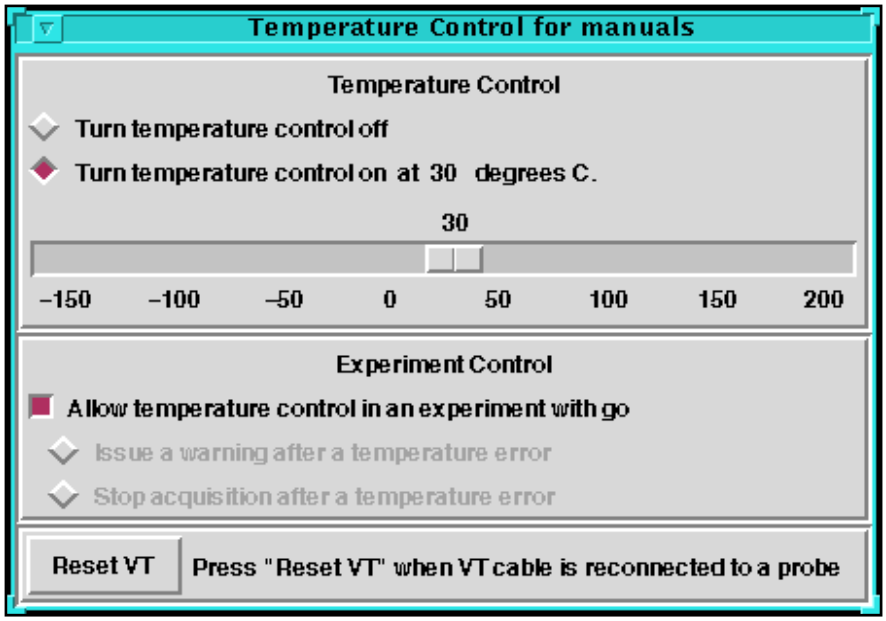

Temperature Control for manuals
Temperature Control
Turn temperature control off
Turn temperature control on at 30 degrees C.
30
–150 –100 –50 0 50 100 150 200
Experiment Control
Allow temperature control in an experiment with go
Issue a warning after a temperature error
Stop acquisition after a temperature error
Reset VT
Press "Reset VT" when VT cable is reconnected to a probe

Figure 61. Temperature Control Window

8.4 Operating Recommendations

The following recommendations should help achieve better VT performance.

- The spectrometer system was designed and tested with a VT gas flow rate of about 10 LPM. Sizable deviation from this rate can result in significant inaccuracy in temperature calibration and reduce the attainable temperature limits.
- Initial cool-down of the exchanger and transfer tubing after the coolant is added increases the initial time required to reach regulation (about 5 to 10 minutes for –40°C with liquid nitrogen). Because this may be longer than the `vtwait` parameter, an `su` command is the best way to start up.
- Below –40°, using dry nitrogen gas for the spinner and bearing air supply avoids moisture and frost buildup on the spinner housing and turbine. Should this happen, the sample spinning can become erratic or stop altogether.
- Every sample has some vertical temperature gradient. Minimize the gradient by *not* filling the sample tube more than about 25 to 32 mm (1 to 1.25 in.), by inserting a vortex plug or glass wool plug in the tube just above the sample solution, and by entering the liquid column to the probe coil center lines. The plug reduces refluxing of the solvent in the upper portion of the tube. Any mass movement, such as refluxing or convection, can seriously degrade resolution and lock stability.
- Above 100°C, use dry nitrogen gas to reduce heater and thermocouple oxidation.
- High-power decoupling adds heat to the sample. The increase in temperature depends on the dielectric of the solution and the power level. Under these conditions, the temperature accuracy under VT control is significantly affected. If necessary, reduce decoupler power and use a more efficient decoupling mode, such as WALTZ-16 or GARP.
- Overnight or long-term unattended VT operation at low temperatures is hampered by the fact that the usual coolant, liquid nitrogen, provides only about 1 to 2 hours of operation on a single fill of the coolant bucket. Some other coolant that lasts longer can

be used if the operating temperature does not require the low temperature of liquid nitrogen. A common alternative is a mixture of dry ice and acetone. Another option is a fluid such as isopropyl alcohol or ethylene glycol cooled indirectly by a refrigerating device. Do not use aromatic, ketone, and chlorinated solvents (including acetone) in the coolant bucket. Such coolant media attack the standard polystyrene bucket.

- The ability of the VT unit to achieve temperature stability is directly affected by the stability of the room temperature. The VT unit compensates for about 80% of external changes (leaving 20% uncompensated for). Thus if the temperature of the room changes by 1°, the sample temperature will change by about 0.2°, which will not be reflected in a change in the numerically displayed temperature. For best results, the room temperature should be made as stable as possible. Any cycling of the temperature due to air conditioning or heating should be accomplished with the shortest possible cycle time and the minimum possible temperature variation.
- High-stability and independence from room temperature can be achieved if the VT controller is equipped with an optional cold junction (CJ) compensator. With the high-stability feature, the VT controller is no longer compensated for room temperature changes, but instead receives its reference from the cold junction devices mounted in the magnet leg. As the CJ compensator reduces the room temperature influences on the system, the influences of the VT gas supply become more apparent. For optimum performance of the CJ compensator, the flow and temperature of the VT gas supply must be as stable as possible.
- A possible setup to help stabilize the VT gas supply is to run the VT gas through a heat-exchanger coil in a water bath at a regulated temperature. For best results, use an ice bath to cool down the VT gas to between 5°C and 10°C, and keep the flow as stable as possible for experiments below 40°C. Generally, for best performance of the VT controller and heater in the probe, the VT gas supply temperature should be a minimum of 10°C below the set temperature.
- For exact determination of sample temperature, a temperature calibration curve must be made for each probe used. All data, such as gas flow, must be noted. Samples of ethylene glycol are used for high-temperature calibration, and samples of methanol are used for low-temperature calibration.

    a. After bringing the sample to the desired temperature and allowing sufficient time for equilibration, obtain a spectrum.

    b. Display two cursors and align them on the two resonances in the spectrum.

    c. If the sample is ethylene glycol, enter `tempcal('e')`; if the sample is methanol, enter `tempcal('m')`.

    d. The temperature is calculated and displayed based on the difference frequency between the cursors.

8.5 VT Controller Safety Circuits

The VT controller includes safety circuits to avoid damage to the heating element and probe. The following error conditions produce an error code in `acqstatus[2]`:

- Open circuit in the thermocouple circuit.
- Open circuit, short circuit, or over-temperature at safety sensor.
- Short circuit or software/microprocessor failure at the output transistor.

Over-temperature at the safety sensor initially turns off the heater. If this method fails to correct the condition within 5 seconds, either the gas flow has been interrupted or an output

transistor failure has occurred, whereupon a protective relay operates, isolating the heater from the control electronics. Failure of any of the sensors also results in this relay operating.

Once the protective relay has operated, the output will remain off. A power-down and power-up cycle of the VT controller is required to release the relay.

The over-temperature circuit can be inadvertently tripped if the VT is started at a below ambient temperature and the temperature is increased greater than 70°C. If the circuit is tripped, reset it by turning the VT off and on, then change to the desired temperature in 50°C steps.

Excessive heat requirements that cause the current to remain near the maximum can also trip the second circuit. Therefore, when using liquid nitrogen for cooling and when operating from 0°C through +25°C, reduce the gas flow rate to between 8 and 9 LPM. Reset will also occur if the VT cable is removed from the probe while the VT is on.

Refer to the VT installation manual for system failure analysis.

8.6 VT Interlock Parameters

The `tin` (temperature interlock) and `vtwait` (VT wait time) parameters check VT operation and stop the experiment if temperature regulation is lost. The `tin` parameter functions much the same as the `in` (lock and spin interlock) parameter:

- If `tin='y'`, the VT regulation light is monitored during the course of the experiment, and if it starts to flash (regulation lost), the current data acquisition is stopped. The acquisition does not resume automatically if regulation is regained.
- If `tin='w'`, the VT regulation light is monitored during the course of the experiment, and if it starts to flash (regulation lost), a warning is generated but acquisition is not stopped.
- If `tin='n'`, the temperature interlock feature is turned off.

For both `tin='y'` and `tin='w'`, the lost regulation causes `werr` processing to occur, thus providing a user-selectable mechanism to respond to VT failure.

The interlock operation does not apply to the cases when VT regulation is temporarily lost as a result of a programmed temperature change in an experiment where `temp` is an array. Also if `tin='y'`, a maximum limit is imposed on the time that the system waits for regulation to be established. This limit is determined by the `vtwait` parameter and is independent of the `pad` parameter. If regulation is not established after the `vtwait` time (normally set to 180 seconds), the system displays the message `VT FAILURE` and does not proceed with the experiment. If the regulation problem is later corrected, the experiment can be resumed with the `go` and `ga` commands.

The VT gas flow has no sensor or interlock. If gas flow stops, the heater is protected by an internal temperature limit sensor that turns off the heater current before the element overheats. Because a loss of gas flow will result in a loss of regulation, any experiment in progress is stopped if `tin='y'`. Only the sample is left unprotected if VT gas stops.

Although `tin` and `vtwait` are not part of a parameter display group, each can be checked and changed in value the same as other parameters (e.g., by entering `tin?` to check the value or by entering `tin='y'` to set the value).

CAUTION: **Do not run unattended a sealed sample of highly volatile materials that must be kept cold to avoid excessive pressure buildup. The undetected loss of VT gas or exchanger coolant could result in the rupture of the sample tube and damage to the probe components.**

Chapter 9. Carousel, SMS, and NMS Automation

Sections in this chapter:

- 9.1 “Carousel Autosampler,” this page
- 9.2 “SMS Autosampler,” page 279
- 9.3 “NMS Autosampler,” page 283
- 9.4 “General Automation Tasks For All Sample Changers,” page 287
- 9.5 “Changing Sample Changers or Serial Ports,” page 293
- 9.6 “Using Gradient Autoshimming with Automation,” page 293
- 9.7 “Automation Run Description,” page 294
- 9.8 “Customizing the Sample Entry Window,” page 299
- 9.9 “Automated Data Acquisition,” page 299
- 9.10 “Automated Data Processing,” page 306
- 9.11 “File Structures in an Automation Run,” page 307

See Chapter 10, “VAST Accessory Operation,” for coverage of the VAST sample changer. This chapter is limited to the SMS, Carousel, NMS, and the ASM-100 sample changers.

The SMS autosampler, Carousel autosampler, NMS, and ASM-100 automatic sample changers mechanically manage the process of removing and inserting samples from a magnet as part of spectrometer operation. With a minor extension of “normal” operating procedures, however, the sample changer can also be integrated into the process of experiment queueing.

The VNMR software can handle as many as 9999 data sets simultaneously in separate experiment files labeled `exp#`, where # is the experiment number. Without a sample changer, these experiment files can be used to queue up a series of NMR experiments (up to nine) on the same sample. By adding a sample changer, these experiments can instead be used for experiments on up to 100 different samples; the system identifies the sample by location number (the `loc` parameter) as one of the parameters in the experiment.

The sample changer is most useful in fully automatic, unattended operation, called *automation mode*, or an *automation run*.

9.1 Carousel Autosampler

This section is organized as follows:

- “Configuring VNMR for the Carousel,” page 268
- “Checking Out the Carousel,” page 269
- “Mounting and Removing the Carousel,” page 271
- “Adjusting the Eject Air,” page 272

- "Loading and Unloading Samples," page 273
- "Running NMR on One Sample at a Time," page 274
- "Running Automated NMR on Up to Nine Samples," page 275
- "Inserting Samples Manually with the Carousel Attached," page 276
- "Carousel Error Codes and Recovery," page 277

A typical use scenario for the Carousel autosampler is as follows:

- Remove the carousel from the driver (Figure 62 is an illustration of the carousel and driver).
- Load samples and turbines.
- Place loaded carousel onto the driver.
- Use the `enter` program to set up the automation run.
- Enter `autogo` to begin the automation run.

If you have not used the Carousel autosampler before or if you want to reacquaint yourself with it, follow the steps in the next three sections.

After you are familiar with the carousel and have attached it to the driver, go to "Adjusting the Eject Air," page 272.

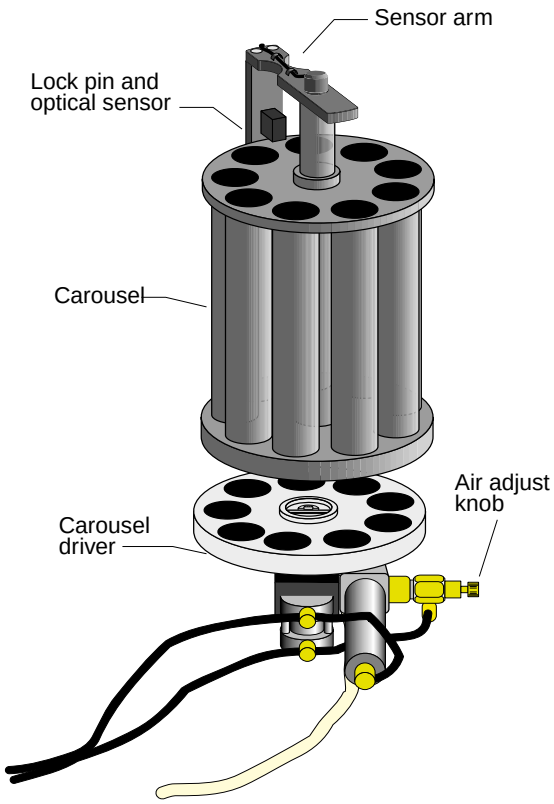


Figure 62. Carousel Autosampler Carousel and Driver

Configuring VNMR for the Carousel

Before you can use the carousel, you must select the carousel in the VNMR configuration window and edit the `enter.conf` file.

1. Log in as **`vnmr1`**.
2. Enter **`config`** in the VNMR input window.
3. In the VNMR Configuration window:
   - Set **Sample Changer** to **Carousel**.
   - Set **Sample Changer Serial Port** to **Port A** or **Port B**, depending on which port is used to connect the sample changer.
   - Click **Exit and Save**.
4. Using a text editor, such as vi, open the file `/vnmr/asm/enter.conf`.
5. Find the line `set loc(max)` at about line 22 in the file. Change the number, if necessary, so that the line is the same as the following:

```
set loc(max) 9
```

6. Save the changes.

VNMR is now configured to use the carousel.

Checking Out the Carousel

Inspecting the carousel before mounting it on the driver helps you understand how it works.

1. With the sensor arm locked (See Figure 63 and Figure 64), look into the carousel tubes. The sensor arm is locked when the arm is positioned between position 9 and position 1 and when the arrow on the knurled knob is pointing to the left or right. The pin of the knurled knob should be in the hole located between positions 9 and 1 on the upper white disk of the carousel (see Figure 64).

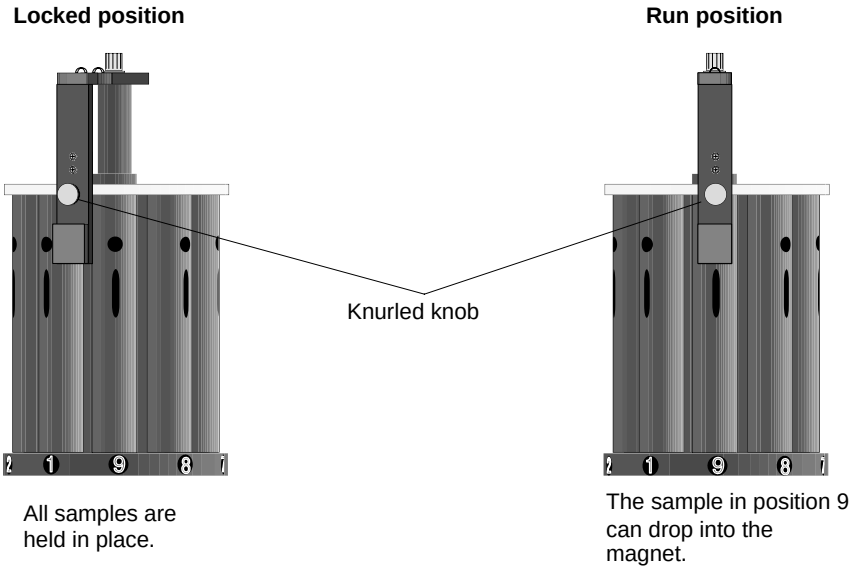

Locked position
Run position
Knurled knob
All samples are held in place.
The sample in position 9 can drop into the magnet.

Figure 63. Carousel with Sensor Arm in Locked and in Run Positions

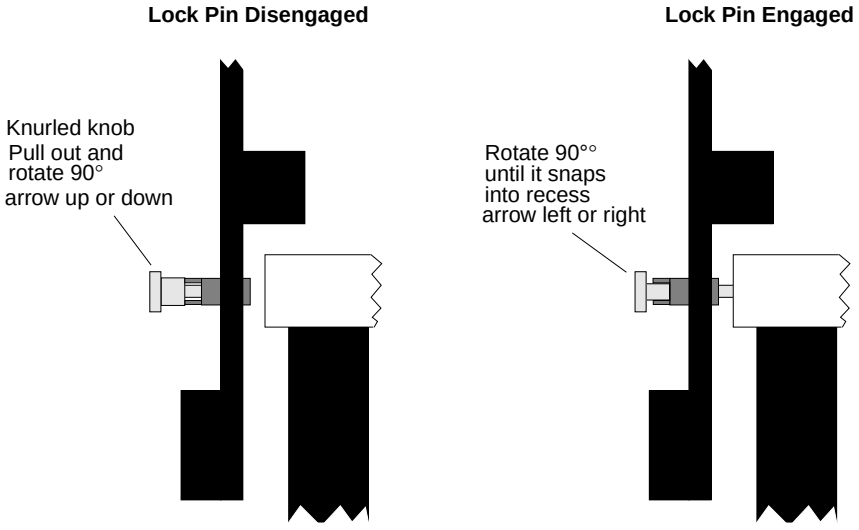


Figure 64. Lock Pin on Sensor Arm Disengaged and Engaged

In *locked mode*, the retaining disk appears in all nine tubes, preventing samples from falling through the bottom. In "run mode," the retaining disk leaves a gap in the tube over the upper barrel, allowing the sample to drop into the magnet.

2. If the sensor arm is locked, disengage the lock pin by pulling out the knurled knob and rotating it 90°. The arrow on the knurled knob now points up or down. See Figure 64. Notice how the sensor arm is free to rotate.
3. Move the sensor arm to the right, from between positions 9 and 1 to directly over position 9. When the carousel is mounted to the driver, the arm only moves between the run and locked positions, shown in Figure 63.
4. Look through the tubes.

   Notice that the white retaining disk inside the carousel has a cutout that aligns with the sensor arm. This cutout allows one sample to drop into the magnet, while the other eight samples rest on the retaining disk.
5. Look at the bottom of the carousel. You see a connector and a groove that fit into the connector and alignment bar on the top of the driver, as shown in Figure 65. Notice the orientation of the connector and the alignment groove and compare this to the driver connector and alignment bar.

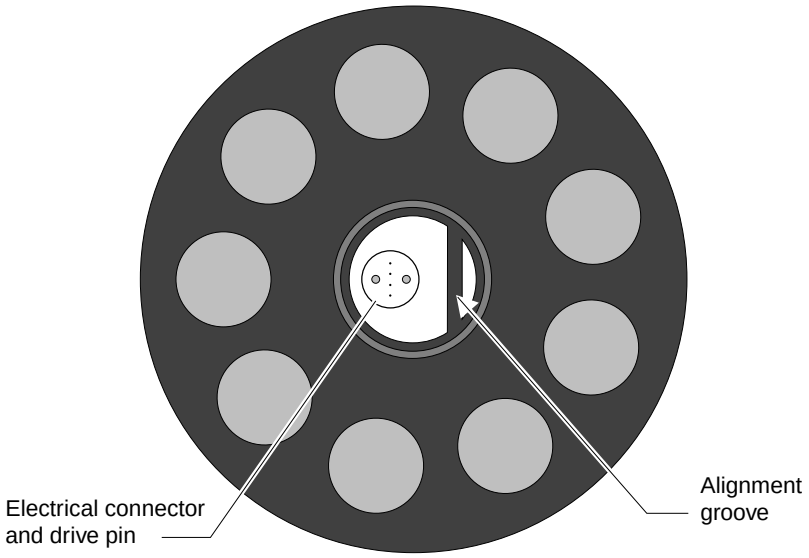


Figure 65. Bottom View of Carousel, Showing Connector and Groove

6. Move the sensor arm to between position 9 and position 1, and then engage the lock pin by rotating it 90°◦ and allowing it to fall into the hole in the upper white disk.
7. Place the empty carousel on the driver according to the instructions in the next section, "Mounting and Removing the Carousel."

After the empty carousel is mounted, it is ready to be used in walk-up mode, as described in "Inserting Samples Manually with the Carousel Attached," page 276.

Mounting and Removing the Carousel

To Mount the Carousel

This procedure describes how to mount the carousel on the driver.

1. Make sure the sensor arm on the carousel is locked in between positions 9 and 1. The groove in the bottom of the carousel (see Figure 65) should be 90° (perpendicular) to position 9.
2. Make sure the driver has position 9 over the upper barrel. If not, use the manual index button to rotate the driver.
3. Align the numbers on the carousel to the numbers on the driver.
4. Push down on the carousel until it seats. This engages the electrical connector and drive pin (see Figure 65).
5. At the console, turn on the eject air.
6. Disengage the lock pin by pulling out the knurled knob and rotating it 90°. The arrow on the knurled knob will point up or down.
7. Move the sensor arm to the right, until it is over position 9. This is the run position. You will notice some resistance as you move the arm.

The carousel is now ready to use for NMR experiments.

To Remove the Carousel

This procedure describes how to remove the carousel from the driver. Position 9 must be over the upper barrel before the carousel is removed.

CAUTION: **Samples could fall through and break if the carousel is not in the locked mode. Before removing the carousel, rotate the carousel so that position 1 is over the upper barrel. The sensor arm must be in the locked position, as shown in Figure 63, with the lock pin engaged and the arrow on the knurled knob pointing to the left or right.**

1. After an automated run is finished, turn on the eject air at the console.
2. Use the manual index button to rotate the carousel, until position 9 aligns with the upper barrel.
3. Move the sensor arm to the left, from run to lock mode (see Figure 63), until the arm stops between position 9 and position 1.

   In the lock position, all the samples rest on the retaining disk.
4. Engage the lock pin by pulling the knurled knob and turning it 90°. The lock pin fits into the hole in the white upper disk of the carousel.

   The locking pin keeps the sensor arm from moving out of the lock position.
5. Remove the carousel by pulling up on the carousel while holding down the driver, rocking gently if necessary. The upper barrel may lift slightly. The sensor arm provides a convenient, central gripping point.

CAUTION: Without the carousel mounted, the eject air pressure is too high and samples may launch out of the magnet when ejected. The procedure in "Inserting Samples Manually with the Carousel Attached," page 276, is recommended for nonautomated work. Otherwise, lower the eject air pressure if you intend to use the magnet without the carousel. Refer to the next section, "Adjusting the Eject Air."

Adjusting the Eject Air

This procedure describes how to adjust the eject air pressure so that the sample and turbine float where the optical sensor on the sensor arm can *see* the sample. Figure 66 shows the optical sensor and the correct sample floating height. The empty carousel should be mounted on the driver as described in the procedure for mounting the carousel on the previous page.

Variation in supply air pressure can cause failures. Adjust the eject air to the lowest level expected from the supply air during a 24-hour period that maintains an acceptable turbine float height for the sensor.

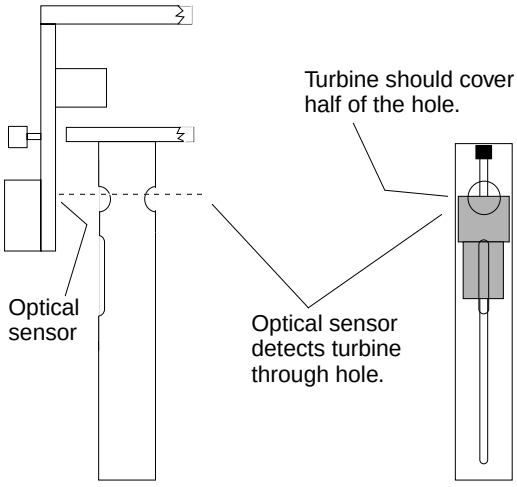


Figure 66. Optical Sensor and Proper Sample Floating Height

1. Make sure the air hoses for body and VT air are attached to the probe and flow meters are set for about 11 cfm.

   Some probes require more eject air than others. If the pressure required to eject the sample and float the turbine adequately exceeds 65 psi, check for the guide hole at the bottom of the probe.

   If your probe has a guide hole in the bottom for a capacitor or inductor stick and if your experiment does not require the stick, plug the guide hole with the provided rubber stopper.

2. In VNMR, turn on the eject air by typing **e** and then pressing Return.

3. With the knurled knob disengaged (see Figure 64), move the sensor arm to the left so that it clicks between positions 9 and 1 (see Figure 63).

4. Place a sample in position 9 of the carousel.

5. Move the sensor arm back in front of position 9.

6. Look through the round hole in the position 9 tube of the carousel.

   The sample should be floating high enough for the optical sensor to see the turbine, as shown in Figure 66. In other words, the turbine should cover at least half of the inside of the round hole.

   - If the turbine is too low, turn up the eject air at the source pressure regulator. If eject air is still insufficient, air may be leaking through a tuning capacitor or inductor guide hole in the bottom of the probe. Use the provided rubber stopper to plug the hole.

- If the turbine is too high, turn down the eject air at the source pressure regulator.

7. After the sample is floating at the correct height, insert the sample using VNMR by typing **i** and pressing Return.

   The sample should drop into the probe. If the sample drops too rapidly, adjust the SLO-DROP air in the magnet leg.

8. Eject the sample again and recheck the height. Adjust the eject air again if necessary.

The Carousel autosampler can now be used for automation. You may have to lower the bearing air to compensate for the higher eject air. Proceed to the next section, which describes how to load samples into the carousel.

Loading and Unloading Samples

The Carousel autosampler provides two methods for loading and unloading samples. The only difference between the two procedures is whether the carousel is left on the driver or removed.

Do not mix turbine types within the carousel, and be sure that eject air is on before the sensor arm is moved from the locked to the run position.

WARNING: **Removing the carousel while reaching over a high-field magnet (such as 400-, 500-, 600-, or 750-MHz) could cause injury if you to lose your balance and fall. Take care when removing the carousel from a high-field magnet.**

To Load and Unload Samples with the Carousel Installed

This procedure describes loading and unloading samples from the carousel while it is still attached to the driver. If you want to remove the carousel before loading and unloading samples, use the next procedure.

1. Enter **e** to turn on the eject air.
2. Move the sensor arm to the left, until it stops between position 9 and position 1.
3. Remove the sample from position 9. Then, remove the rest of the samples.
4. With the eject air still on, insert the samples into the carousel in any order. Be sure to remember which samples are in which positions.
5. Move the sensor arm back to the right, until it aligns with position 9.

   The sample in position 9 will float. Now is a good time to check sample float height. Adjust eject air if necessary, as described in “Adjusting the Eject Air,” page 272.

The Carousel autosampler can now be used for NMR.

To Remove the Carousel and Load Samples

This procedure describes removing the carousel then loading or unloading samples from the carousel.

1. Enter **e** in VNMR to turn on the *eject* air.
2. If position 9 is not already aligned over the upper barrel, use the manual index button to index the carousel to position 9, or enter **loc=9 change** in VNMR.

3. Move the sensor arm to the locked position and engage the lock pin as follows. Carousel must be in the lock position when removed; otherwise, samples could fall through the carousel and remounting the carousel would be extremely difficult.
   a. Move sensor arm to the left, until it stops between position 9 and position 1.
   b. Engage the lock pin by rotating the knurled knob 90°, until the arrow points left or right.
4. Remove the carousel by pulling up on the carousel while holding down the driver, rocking gently if necessary. The upper barrel may lift slightly. The sensor arm provides a convenient, central gripping point.
5. Place the carousel on a table.
6. Remove the samples from the carousel.
7. Load the new samples into the carousel.
8. Mount the carousel on the driver. Match the sample position numbers on the carousel to the numbers on the driver. Press down on the carousel until it seats.
9. Enter **e** in VNMR to turn on the eject air.
10. Disengage the lock pin and move the sensor arm to the right, until it aligns with position 9.

    The sample in position 9 should float at the correct height. If not, adjust the eject air as described in "Adjusting the Eject Air," page 272.

The Carousel autosampler can now be used for NMR.

Running NMR on One Sample at a Time

You can run NMR on any individual sample in the carousel using the `loc` parameter and the `change` command.

1. Make sure the carousel is loaded with nine samples and is installed on the carousel driver, as described in previous sections.
2. Enter **e** in VNMR to turn on the eject air.
3. Disengage the lock pin and then move the sensor arm to the right until it stops in front of position 9.
4. Check that the eject air pressure is sufficient to float the turbine/sample to the correct height. If not, adjust the eject air as given in "Adjusting the Eject Air," page 272.
5. Using the manual index button, rotate the carousel through all positions. Check the turbine/sample float height for each position. Stop when position 9 is over the upper barrel.
6. Enter **`loc=1 change`** to change to position 1.

   The carousel does the following:

   - The message `exp1: Experiment started` appears on the Sun computer.
   - The carousel rotates a half position (in this case between positions 9 and 1).
   - The carousel rotates to position 1 and inserts the sample.
   - The message `exp1:Setup complete` appears on the Sun computer.
7. Run the desired NMR experiment.
8. Enter **`loc=2 change`** to change to position 2. Run an experiment if desired.

Running Automated NMR on Up to Nine Samples

To run a series of NMR experiments on some or all of the samples in the carousel, load up to nine samples into the carousel and use the `enter` program to set up the automation run.

Begin the automation run with the sample in position 9 inserted into the magnet. When the automation run begins, the Carousel ejects the sample from location 9, rotates to location 1, and inserts the sample from location 1.

After an automation run finishes, the last sample location run is left in the magnet.

1. Prepare your samples and have a list of samples and experiments ready.
2. Load up to 9 samples as described in "Loading and Unloading Samples," page 273.
3. If not already done, insert the sample from location 9.

   When the automation run begins, the Carousel ejects the sample from location 9, rotates to location 1, and inserts the sample from location 1.
4. Enter **`cd`** to change to your home directory.
5. Type **`enter`** in the VNMR input window.

   You are prompted for a directory name that stores the information set by the `enter` program.

   You can also use the command `enter('abc')` creates a directory named `abc`. In the `abc` directory, there is a file named `abc`, which contains experiment information. Also in the directory is a directory named `abc.macdir`, which contains *GLIDE*-related information for an automation run.

   The Sample Entry Form window (see Figure 67) appears.

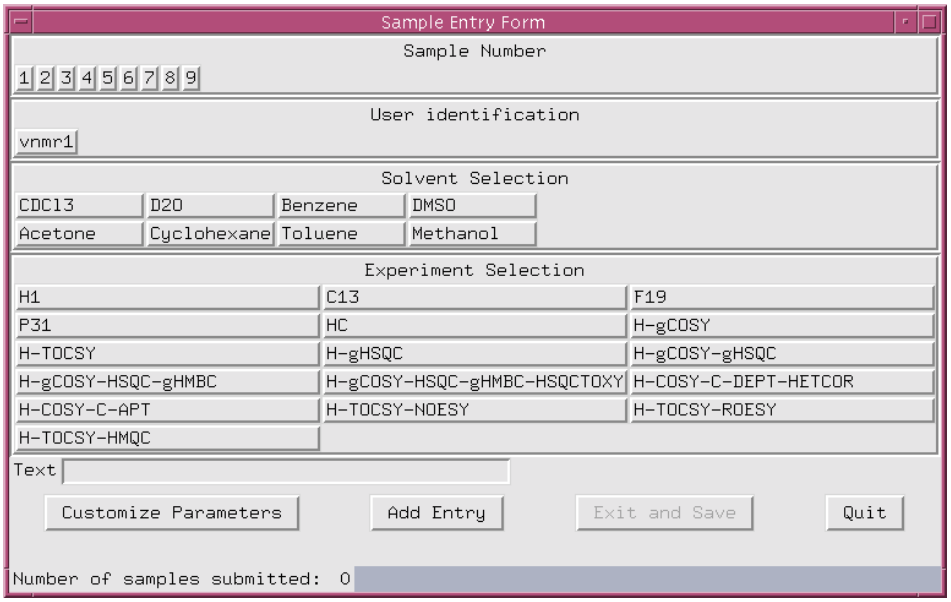
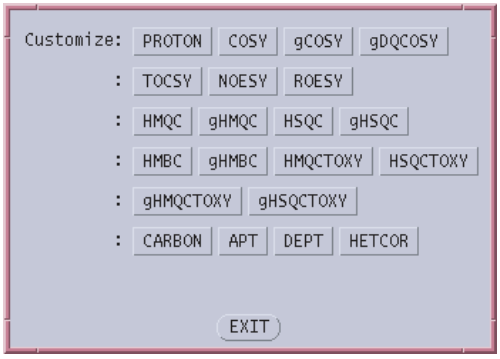


Figure 67. Sample Entry Form Window for the Carousel Autosampler

6. Fill in the Sample Entry Form window.
   - **Sample Number** – select one or more sample locations on which to run experiments.

- **User Identification** – select the user.
- **Solvent Selection** – select the solvent for the chosen locations(s).
- **Experiment Selection** – select one or more experiments for the chosen locations(s).
- **Text** – enter information about the experiment, if desired.
- To customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles, click the **Customize Parameters** button.

  Click on the item you want to customize. A new window opens and provides appropriate parameters to change.



- When finished with this location (or locations), click **Add Entry**.

When you are done adding entries, click **Exit and Save**.

7. To start the automation run, enter **`autogo`**.
   - When the system asks `Location of automation queue`, enter the directory name you used in step 5.
   - When the system asks Location of automation data, accept the default or enter a new directory name.

   You can also enter `autogo('MySamples')` or `autogo('MySamples','AutoRun_621')`.

   The data is stored in the automation directory, with the usual automation-style file names (as specified by `autoname` if desired). The acquisition takes place in background within VNMR, allowing the user complete freedom to process other dataset in any desired experiment location.
8. If any error messages appear, refer to "Carousel Error Codes and Recovery," page 277 for an explanation.
9. To monitor the automation run, enter **`status`** to bring up the Status window. Refer to "Monitoring an Automation Run," page 289 for more details.
10. Return later to retrieve your results.

    For each experiment, a plot is created and a FID file is saved in the automation directory (e.g., `AutoRun_621`).

    The FID files and the `fid` extension are the data files collected during the acquisition run. Normally, as data is collected, the FIDs are placed into the automation directory with the name `xxyy.fid`, where `xx` is the sample location number and `yy` is an "experiment number" on that particular sample (`1` through `n`, where `n` experiments have been run on the same sample).

Inserting Samples Manually with the Carousel Attached

Since the eject air is higher than normal, the carousel should remain attached to the driver, even when empty. Therefore, the Carousel autosampler provides a mode that allows you to

insert and eject samples when the carousel is not being used for automation. In other words, if you want to run one sample at a time, as in normal operation, you should do so with the carousel in place.

This procedure describes how to insert and eject samples, one at a time, through the carousel. The carousel must be on the magnet with no sample in position 9.

1. Enter **e** in VNMR to turn on eject air.
2. Make sure no sample is in position 9, and make sure position 9 is aligned over the upper barrel.
3. If the sensor arm is over position 9, move it to the left until it stops between position 9 and position 1, as shown in Figure 68. You will notice some resistance moving the arm. Do not engage the locking pin.

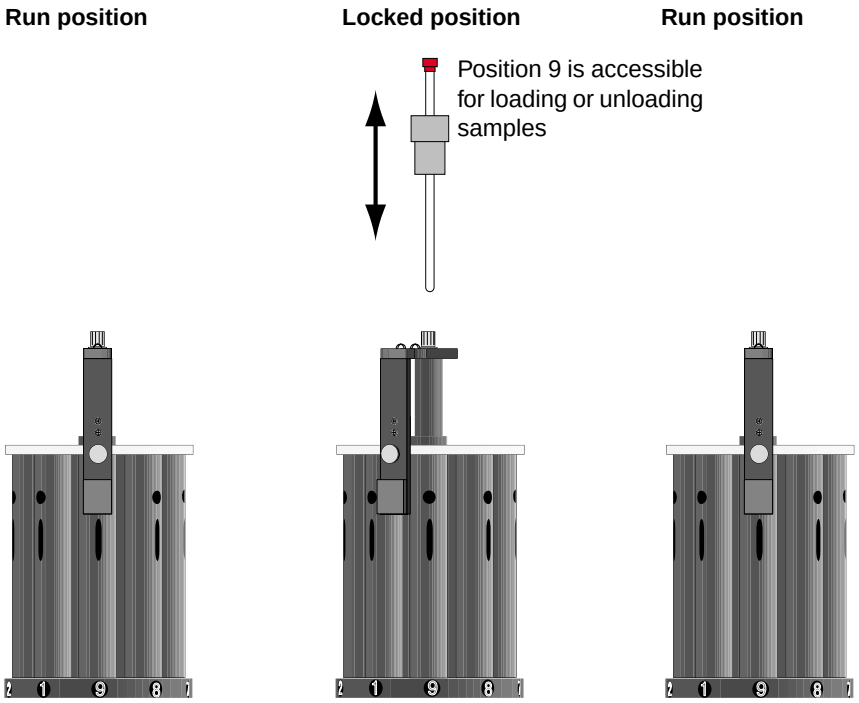


Figure 68. Manually Loading and Unloading Samples Through Position 1

4. Place your sample and turbine in position 9.
5. With eject air still on, rotate the sensor arm back to position 1.

   The sample can be inserted and ejected, as in normal, nonautomated operation.
6. To remove the floating sample from the magnet, rotate the sensor arm to the left until it stops between position 9 and position 1. Then lift the sample straight up and out.

Carousel Error Codes and Recovery

This section defines the error codes and suggests ways to recover from the errors. The following messages are common to all Varian autosamplers. The accompanying descriptions and remedies, however, refer to the Carousel autosampler.

Sample changer arm unable to move sideways during retrieve.

Index air is too low. Check and adjust the index air regulator to increase the air pressure to 38 psi.

Sample tray size is not consistent.

System is configured with wrong tray size. Open the CONFIG window and make sure Sample Tray Size is set to 9.

Invalid sample number during retrieve.

Location value too high for Carousel and `traymax` configuration incorrect. Only positions 1 through 9 are valid for the Carousel. Open the CONFIG window and make sure Sample Changer is set to Carousel (or Sample Tray Size is set to 9).

Sample out of range during automatic retrieve.

Sample tube is floating too low. Slightly increase the system eject air one of the following ways:

- Increase the cooling air flow.
- Make sure the body air and VT air hoses are connected to the probe and the flow meters are set to 11 cfm.
- Increase supply air pressure.
- Plug the capacitor/inductor guide hole in the bottom of the probe with the provided rubber stopper.

Robot arm failed to find home position during retrieve.

Carousel is not in an indexed position. Adjust the air adjust knob on the driver so that the carousel rotates properly. The micro switch should insert fully into the recess on the underside of the white disk of the driver.

- If the carousel rotated too far, turn the air adjust knob 1/16 of a turn at a time in the clockwise direction. Test using the manual index button (press and hold the manual index button and wait for motion).
- If the carousel does not rotate far enough, turn the air adjust knob 1/16 of a turn in the counter-clockwise direction. Test using the manual index button (press and hold the manual index button and wait for motion).

Air supply to sample changer failed during retrieve.

No tube or sample is detected. Verify that a sample is in the magnet or carousel.

- If no sample is present, do an eject and place a sample in the carousel. Then, continue to the next sample.
- If a sample is present, check the eject air and adjust if necessary, as described in “Adjusting the Eject Air,” page 272.
- If the sample sticks in the lower part of the barrel, be sure the carousel is fully seated on the driver.

9.2 SMS Autosampler

This section is organized as follows:

- "Configuring VNMR for the SMS Autosampler," this page
- "Preparing Sample Tubes," page 280
- "Running NMR on One Sample at a Time," page 280
- "Running Automated NMR," page 281
- "SMS Error Codes and Recovery," page 282

The SMS (Sample Management System) autosampler accessory (illustrated in Figure 69) is an electromechanical, robotic device designed to automatically insert and retrieve samples from the bore of the magnet. The system is designed for completely automatic, unattended operation and can handle from 1 to 100 samples.

When installed, the robot is attached to a table next to the magnet, where the robot is calibrated and "taught" the magnet and sample rack positions.

Two sample tray configurations are available:

- A 50-sample tray accommodates 5-mm and 10-mm sample tubes.
- Two 50-sample trays accommodate one hundred 5-mm and 10-mm sample tubes.

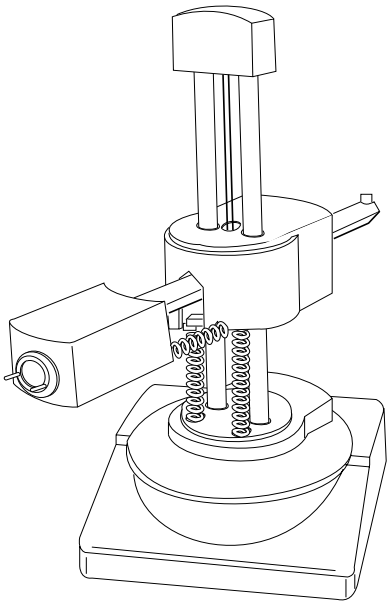


Figure 69. SMS Autosampler

Each sample position is individually encoded so that the computer can address a position either randomly or sequentially. In addition, sample locations are staggered to improve the identification of each sample position. Sample zero is a default position accessible by the autosampler for various error recovery scenarios, including power failures.

The autosampler and the acquisition system communicate through a standard RS-232C serial link at 9600 baud. Time between sample changes is about 35 seconds for a complete cycle—retrieve the sample from the magnet, return it to the sample tray, locate and pick up the next sample, and deliver it to the magnet.

Configuring VNMR for the SMS Autosampler

Before you can use the SMS, VNMR must be configured as follows:

1. Log in as `vnmr1`, and enter **`config`**.
2. In the VNMR configuration window, make the following changes:
   - **Sample Changer** to **SMS 50 Sample** or **SMS 100 Sample**.
   - **Sample Changer Serial Port** to **Port A** or **Port B**, depending on which one of the Sun serial ports is used for the sample changer.
   - Click **Exit and Save**.

3. To get the 100 sample positions (for dual 50-sample trays) to appear in the Sample Entry Form window, use a text editor, such as vi, to edit the file `/vnmr/asm/enter.conf`.
4. Find the line `set loc(max)` at about line 22 in the file. Change the number, if necessary, so that the line is the same as the following:
   `set loc(max) 100`
5. Save the changes.

VNMR is now configured to use the SMS autosampler.

Preparing Sample Tubes

Every automation run starts with sample preparation. Tube lengths are constrained by the geometry of the sample changer to be either 7 in. or 8 in. tubes.

Do not attempt to use longer (e.g., 9 in.) tubes because the top of such a tube may come into contact with the bottom of a tube being held by the gripper arm.

Positioning the sample in the spinner and adjusting the height of the liquid in the tube are both done the same way as in manual operation, but position and sample height are much more critical in automated operation. The more precise your tube position and the more you are careful to adjust each sample to exactly the same height, the fewer problems you will have with automatic locking and shimming, and the less time you will have to take for these operations to occur.

If at all possible, fill tubes to a height of 50 mm (about 2 inches).

Running NMR on One Sample at a Time

You can run NMR on any individual sample in the sample tray using the `loc` parameter and the `change` command.

1. Prepare the samples you want to run and place them in the sample tray. It might be helpful to write down the location of each sample.
2. Enter **`loc=# change`**, where # is the location of the sample you want to run.

   For example, to insert the sample at location 3, enter **`loc=3 change`**.

   The SMS does the following:

   - The message `exp1: Experiment started` appears on the Sun computer.
   - The SMS arm retrieves the sample from the specified location.
   - The eject air turns on, the SMS inserts the sample, and the sample inserts into the probe.
   - The message `exp1:Setup complete` appears on the Sun computer.
3. Run the desired NMR experiment.
4. Enter **`loc=# change`**, where # is the location of the next sample you want to run.

   The previous sample is removed from the magnet and returned to its location. The next sample is retrieved from the tray and inserted into the magnet.

   Run an experiment if desired.

Running Automated NMR

To run a series of NMR experiments on some or all of the samples in the sample tray(s), load up to 50 samples for a single 50-sample tray, or up to 100 samples for dual 50-sample trays, and use the `enter` program to set up the automation run.

1. Prepare the samples you want to run and place them in the sample tray. It might be helpful to write down the location of each sample.
2. Enter **`cd`** to change to your home directory.
3. Type **`enter`** in the VNMR input window.

   You are prompted for a directory name to store the information set by `enter`.

   You can also invoke the command `enter(`*`directory`*`)` to create a directory. For example, `enter('abc')` creates a directory named `abc`. In `abc`, a file named `abc` contains experiment information. Also in the `abc` directory, the directory `abc.macdir` contains *GLIDE*-related information for an automation run.

   The Sample Entry Form window (see Figure 70) appears.

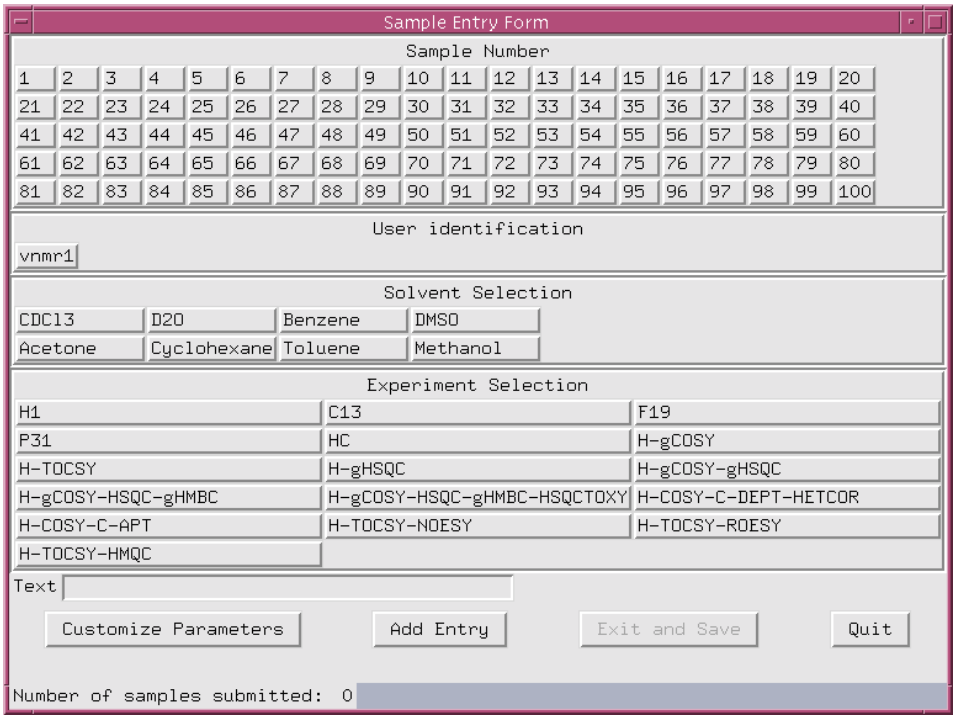
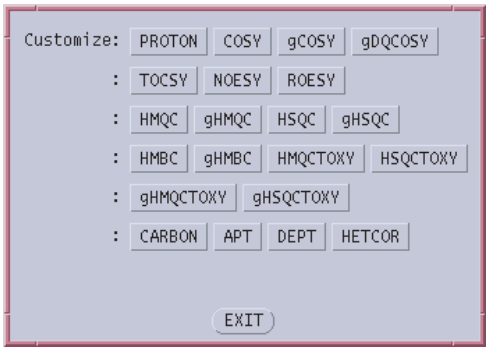

Sample Entry Form
Sample Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
User identification
vnmr1
Solvent Selection
CDCl3 D2O Benzene DMSO
Acetone Cyclohexane Toluene Methanol
Experiment Selection
H1 C13 F19
P31 HC H-gCOSY
H-TOCSY H-gHSQC H-gCOSY-gHSQC
H-gCOSY-HSQC-gHMBC H-gCOSY-HSQC-gHMBC-HSQCTOXY H-COSY-C-DEPT-HETCOR
H-COSY-C-APT H-TOCSY-NOESY H-TOCSY-ROESY
H-TOCSY-HMQC
Text
Customize Parameters
Add Entry
Exit and Save
Quit
Number of samples submitted: 0

Figure 70. Sample Entry Form Window (100 samples) for the SMS Autosampler

4. Fill in the Sample Entry Form window.
   - **Sample Number** – select one or more sample locations on which to run experiments.
   - **User Identification** – select the user.
   - **Solvent Selection** – select the solvent for the chosen location(s).
   - **Experiment Selection** – select one or more experiments for the chosen location(s).

- **Text** – enter information about the experiment, if desired.
- To customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles, click the **Customize Parameters** button.

  Click on the item you want to customize. A new window opens and provides appropriate parameters to change.



- When finished with this location (or locations), click **Add Entry**.

When you are done adding entries, click **Exit and Save**.

5. To start the automation run, enter **`autogo`**.
   - When the system asks `Location of automation queue`, enter the directory name you used in step 3.
   - When the system asks Location of automation data, accept the default or enter a new directory name.

   You can also enter `autogo('MySamples')` or `autogo('MySamples','AutoRun_621')`.

   The data is stored in the automation directory, with the usual automation-style file names (as specified by `autoname` if desired). The acquisition takes place in background within VNMR, allowing the user complete freedom to process other dataset in any desired experiment location.
6. If any error messages appear, refer to “SMS Error Codes and Recovery” for an explanation.
7. To monitor the automation run, enter **`status`** to bring up the Status window. Refer to “Monitoring an Automation Run,” page 289, for more details.
8. Return later to retrieve your results.

   For each experiment, a plot is created and a FID file is saved in the automation directory (e.g., `AutoRun621`).

   The FID files the `fid` extension and are the data files collected during the acquisition run. Normally, as data is collected, the FIDs are placed into the automation directory with the name `xxyy.fid`, where `xx` is the sample location number and `yy` is an “experiment number” on that particular sample (`1` through `n`, where `n` experiments have been run on the same sample).

SMS Error Codes and Recovery

This section contains a list of the error messages returned by the spectrometer when there is a problem. Under each error message is an explanation and correction.

General Operation Errors

The following errors indicate a problem with the general operation of the autosampler.

- `ERROR 96`

SMS autosampler is plugged into the RS-232C but not turned on.

- `ERROR 97`
  SMS autosampler is not plugged into the RS-232C connector.
- `ERROR 98`
  If you receive ERROR 98 while executing `loc=x` or `LOC=X` change command or while attempting an automation run, reinitialize the System V Controller by turning its power off and then on. Wait for the beep to indicate that it is ready. Try the operation from the console.
- `ERROR 99`
  SMS autosampler is not responding to issued commands.

Sample Removal Errors

The following errors indicate a problem with removing samples from the sample tray.

- `ERROR 01 NO SAMPLE`
  The gripper has been commanded to close with no sample because the fingers close completely. This may be a normal indication, for example, when executing `R0` (retrieve sample 0) to check the magnet for a sample. If a fault occurs, the sample switch may need adjustment.
- `ERROR 05 INVALID SAMPLE NUMBER`
  The sample number exceeds the positions available on the tray. This error message appears if you attempt to load sample 0.
- `ERROR 07 GRIPPER ABORT`
  The gripper is compressed and unable to operate, usually because it has contacted another surface.
- `ERROR 13 ILLEGAL COMMAND` (shutdown?)
  An illegal character has been received. This is a normal response to any command except E (energize) or H (help) when in the shutdown mode.

Sample Loading Errors

If a sample removal error occurs during sample loading, the same error message will appear on the monitor. However, the error number will be different; the error number will equal the corresponding sample removal error number plus 20. For example, if a gripper abort error occurs during sample loading, error number 27 (7 + 20) will appear on the monitor.

9.3 NMS Autosampler

This section is organized as follows:

- “Configuring VNMR for the NMS Autosampler,” page 284
- “Running NMR on One Sample at a Time,” page 285
- “Running Automated NMR,” page 285

The Nano Multisampler (NMS) automates the steps required to position a 4-mm rotor into the bore of a magnet for NMR analysis. The rotor is spun at a magic angle of 54.7° in a stator within the body of a Nano probe. The probe is subsequently lifted into the bottom bore of an NMR magnet.

The NMS is comprised of the following main units:

- *Probe elevator* – Attaches to the shim coil flange at the bottom of the magnet, in place of a probe. This unit lifts the probe into the magnet bore and lowers the probe out of the magnet bore.
- *Probe flange* – Fits onto the probe shield and then snaps into the elevator.
- *NMS Controller* – Sits on the floor near the magnet. The NMS controller provides serial (RS-232) communications between the NMS and the NMR host computer. The NMS controller also provides signal and pneumatic connections to the probe elevator and the carousel.
- *Carousel* – Sits on the floor aligned with the tilted probe. The carousel holds a 48-sample rack and replaces the sample in the probe with a new one from the rack.

The probe elevator and NMS controller can be used together to provide a semi-automated means of inserting a Nano probe into the NMR magnet. The addition of the carousel provides an automated means for loading each of 48 rotors into a probe.

The NMS window, shown in Figure 71, provides general controls for installing and operating the NMS sample changer.

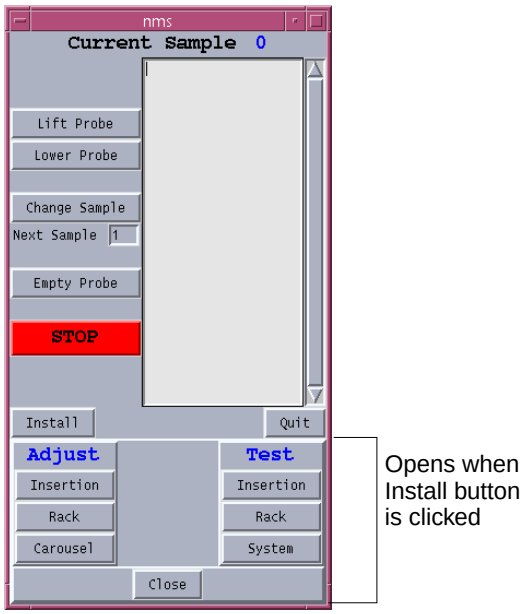


Figure 71. NMS Window

Before Using NMS

Before the NMS autosampler is used, the probe elevator must be attached to the shim coil and the carousel must be properly positioned. *No sample can be in the probe when you begin using the NMS autosampler.*

Configuring VNMR for the NMS Autosampler

Before you can use the NMS, make sure VNMR is configured as follows:

1. Log in as `vnmr1`, enter **config**.
2. In the VNMR configuration window, make the following selections:
   - **Sample Changer** to **NMS**.
   - **Sample Changer Serial Port** to **Port A** or **Port B**, depending on which one of the Sun serial ports is used for the sample changer.
   - Click **Exit and Save**.
3. To get the proper number of sample positions to appear in the Sample Entry Form window, use a text editor, such as vi, to edit the file `/vnmr/asm/enter.conf`.

   Find the line `set loc(max)` at about line 22 in the file. Change the number, if necessary, so that the line is the same as the following:
   ```
   set loc(max) 48
   ```
4. Save the changes.

VNMR is now configured to use the NMS.

Running NMR on One Sample at a Time

You can run NMR on any individual sample in the sample tray using the `loc` parameter and the `change` command.

1. Prepare the samples you want to run and place them in the sample tray. It might be helpful to write down the location of each sample.
2. Enter **`loc=# change`**, where # is the location number of the sample you want to run. For example, to insert the sample at location 3, you would enter **`loc=3 change`**.

   The NMS does the following:

   - The message `exp1: Experiment started` appears on the Sun computer.
   - The probe elevator lowers the probe out of the magnet and tilts the probe.
   - The suction cup arm on the carousel unit removes the sample from the probe stator and returns it to the tray, the sample tray rotates to the desired location, and the suction cup arm retrieves the desired sample and places it into the probe stator.
   - The message `exp1:Setup complete` appears on the Sun computer.
3. Run the desired NMR experiment.
4. Enter **`loc=# change`**, where # is the location of the next sample you want to run.

   The previous sample is removed from the magnet and returned to its location. The next sample is retrieved from the tray and inserted into the magnet.

   Run an experiment if desired.

Running Automated NMR

To run a series of NMR experiments on some or all of the samples in the sample tray, load up to 48 samples, and use the `enter` program to set up the automation run.

1. Prepare the samples you want to run and place them in the sample tray. It might be helpful to write down the location of each sample.
2. Enter **`cd`** to change to your home directory.
3. Type **`enter`** in the VNMR input window.

   You are prompted for a directory name to store the information set by `enter`.

   You can also invoke the command `enter(`*`directory`*`)` to create a directory. For example, `enter('abc')` creates a directory named `abc`. In `abc`, a file named `abc` contains experiment information. Also in the `abc` directory, the directory `abc.macdir` contains *GLIDE*-related information for an automation run.

   The Sample Entry Form window (see Figure 72) appears.
4. Fill in the Sample Entry Form window.
   - **Sample Number** – select one or more sample locations on which to run experiments.
   - **User Identification** – select the user.
   - **Solvent Selection** – select the solvent for the chosen locations(s).

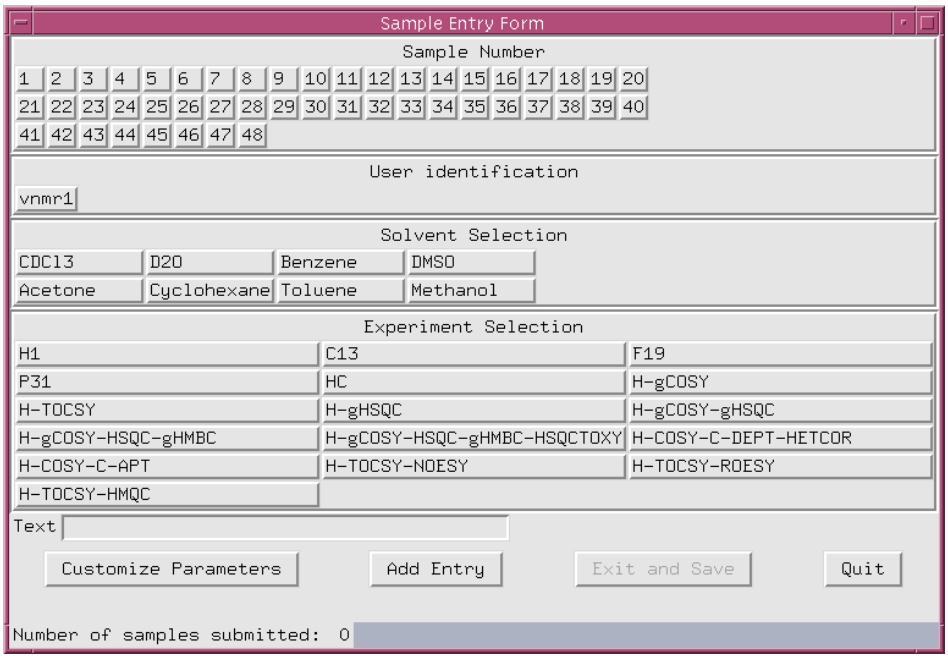
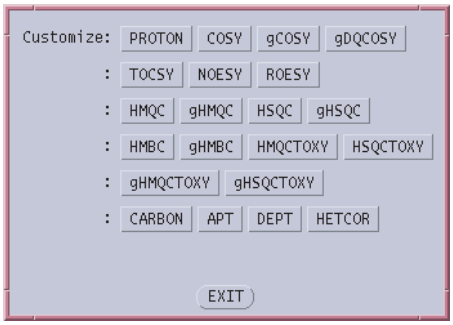

Sample Entry Form
Sample Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48
User identification
vnmr1
Solvent Selection
CDCl3 D2O Benzene DMSO
Acetone Cyclohexane Toluene Methanol
Experiment Selection
H1 C13 F19
P31 HC H-gCOSY
H-TOCSY H-gHSQC H-gCOSY-gHSQC
H-gCOSY-HSQC-gHMBC H-gCOSY-HSQC-gHMBC-HSQCTOXY H-COSY-C-DEPT-HETCOR
H-COSY-C-APT H-TOCSY-NOESY H-TOCSY-ROESY
H-TOCSY-HMQC
Text
Customize Parameters
Add Entry
Exit and Save
Quit
Number of samples submitted: 0

Figure 72. Sample Entry Form Window for the NMS Autosampler

- **Experiment Selection** – select one or more experiments for the chosen locations(s).
- **Text** – enter information about the experiment, if desired.
- To customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles, click the **Customize Parameters** button.

  Click on the item you want to customize. A new window opens and provides appropriate parameters to change.


Customize: PROTON COSY gCOSY gDQCOSY
: TOCSY NOESY ROESY
: HMQC gHMQC HSQC gHSQC
: HMBC gHMBC HMQCTOXY HSQCTOXY
: gHMQCTOXY gHSQCTOXY
: CARBON APT DEPT HETCOR
EXIT

- When finished with this location (or locations), click **Add Entry**.

When you are done adding entries, click **Exit and Save**.

5. To start the automation run, enter **`autogo`**.
   - When the system asks `Location of automation queue`, enter the directory name you used in step 3.
   - When the system asks `Location of automation data`, accept the default or enter a new directory name.

   You can also enter `autogo('MySamples')` or `autogo('MySamples','AutoRun_621')`.

The data is stored in the automation directory, with the usual automation-style file names (as specified by `autoname` if desired). The acquisition takes place in background within VNMR, allowing the user complete freedom to process other dataset in any desired experiment location.

6. If any error messages appear, refer to "Carousel Error Codes and Recovery," page 277, for an explanation.
7. To monitor the automation run, enter **`status`** to bring up the Status window. Refer to "Monitoring an Automation Run," page 289, for more details.
8. Return later to retrieve your results.

   For each experiment, a plot is created and a FID file is saved in the automation directory (e.g., `AutoRun_621`).

   The FID files and the `fid` extension are the data files collected during the acquisition run. Normally, as data is collected, the FIDs are placed into the automation directory with the name `xxyy.fid`, where `xx` is the sample location number and `yy` is an "experiment number" on that particular sample (`1` through `n`, where `n` experiments have been run on the same sample).

9.4 General Automation Tasks For All Sample Changers

The procedures in this section (listed below) and general automation tasks that can apply to all sample changers:

- "Preparing and Initiating an Automation Run," this page
- "Setting Up an Automation Run for Multiple Users," page 288
- "Monitoring an Automation Run," page 289
- "Using Sample Changers in Continuous Walkup Mode," page 291
- "Adding Samples to an Automation Run in Progress," page 292

Preparing and Initiating an Automation Run

1. Prepare your samples and have a list of samples and experiments ready.
2. Insert the samples in the carousel or sample tray (an automation run should not be in progress).
3. Enter **`cd`** to change to your home directory.
4. Type **`enter`** in the VNMR input window.

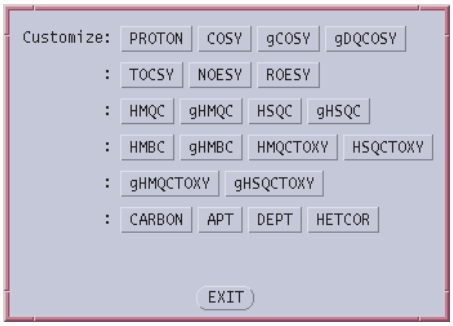
   You are prompted for a directory name to store the information set by `enter`.

   You can also invoke the command `enter(`*`directory`*`)` to create a directory. For example, `enter('abc')` creates a directory named `abc`. In `abc`, a file named `abc` contains experiment information. Also in the `abc` directory, the directory `abc.macdir` contains *GLIDE*-related information for an automation run.

   The Sample Entry Form window appears (an example is shown in Figure 72).
5. Fill in the Sample Entry Form window:
   - **Sample Number** – select one or more sample locations on which to run experiments.
   - **User Identification** – select the user.

- **Solvent Selection** – select the solvent for the chosen peak(s).
- **Experiment Selection** – select Autoscout, Autowettocsy, or both for the chosen peaks.
- **Text** – enter information about the experiment, if desired.
- **Customize Parameters** – Click this button to customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles.

  Click on the item you want to customize. A new window opens and provides appropriate parameters to change.



- When finished with this peak (or peaks), click **Add Entry**.

6. Repeat step 5 for each sample.
7. Click on the **Exit and Save** button when you are finished.
8. Enter **`autogo('mydata','myauto')`** to start the automation run.

   The command `autogo('mydata','myauto')` creates a new automation directory (called `myauto`) in the users home directory and submits the experiment information contained in the `mydata` directory to the new `myauto` directory.
9. To monitor the automation run, enter **`status`** to bring up the Status window. Refer to "Monitoring an Automation Run," page 289, for more details.
10. Return later to retrieve your results.

    For each experiment, a plot is created and a FID file is saved in the automation directory (e.g., `myauto`).

    The FID files and the `fid` extension are the data files collected during the acquisition run. Normally, as data is collected, the FIDs are placed into the automation directory with the name `xxyy.fid`, where `xx` is the sample location number and `yy` is an "experiment number" on that particular sample (`1` through `n`, where `n` experiments have been run on the same sample).

Setting Up an Automation Run for Multiple Users

Automation runs can be set up for multiple users.

The sample entry process might typically occur during the course of the day (assuming a common case of open access operation during the day, and sample changer run at night). One or more operators can use the `enter` program to create a file of information about the samples and experiments they wish to run.

All the information created by individual users must reside in a single file before the automation run is started. To get this final, single file, multiple users could do one of the following:

- Enter information into a single file consecutively.
- Enter information into different files simultaneously. The system operator must eventually merge the various files into a single file using a text editor.

Below are two examples of how this could be implemented.

Example of Using a Common File

1. The system operator `vnmr1` creates an empty file in a location where everyone can access it. A simple way to create a file is to log in as `vnmr1` and enter the command **`touch /vnmr/samples`**. Also enter **`chmod a+w /vnmr/samples`** to give the file write access to all. Notify each user about the name of the file and give them a time to use it.
2. To add information to the agreed upon text file, each user runs the `enter` program by entering **`enter('/vnmr/samples')`**, clicking on the **Add Entry** and then the **Exit and Save** buttons.
3. The sample changer operator supplies the name of the common file as the first argument to `autogo`, as shown in step 8 of the procedure "Preparing and Initiating an Automation Run," page 287.

Example of Using UNIX Mail to Gather Files

1. Each user enters the command **`enter mydata`** from the UNIX level, on any computer on the network that has VNMR software on it.
2. Next, the user enters sample information as noted in step 5 in "Preparing and Initiating an Automation Run," page 287, and then mails a message to the sample changer operator, like this:
   ```
   mail bill
   Subject: sample changer
   my sample changer info is in file /home/jim/mydata
   ```
   Press Control-D to end the message.
3. The sample changer operator enters the following command (on one line):
   ```
   cat /export/home/jim/samples \
   export/home/george/samples \
   export/home/expts > /export/home/vnmr1/asm
   ```
   This command combines files from all users and must be typed on a single line. Note that the order in which experiments are entered into this file will determine the order in which they are run.
4. The sample changer operator, using a UNIX text editor such as `vi`, edits the file just created, changing the sample location numbers to correspond to actual location numbers as samples are entered into the tray (as an alternative, the operator might preassign particular locations to certain users, and require them to have entered the correct numbers themselves in step 1).
5. The sample changer operator supplies the name of the common file as the first argument to `autogo`, as shown in step 8 of the procedure "Preparing and Initiating an Automation Run," page 287.

Monitoring an Automation Run

During an automation run, you can check to see if an experiment is queued, active, or completed.

- To monitor an automation run, enter **`status`** in the VNMR input window.

  The Sample Status window appears, as shown in Figure 73.

  At the top of the Sample Status window, a table summarizes how many samples are queued, how many are active, etc. The color coding in the table identifies the status of

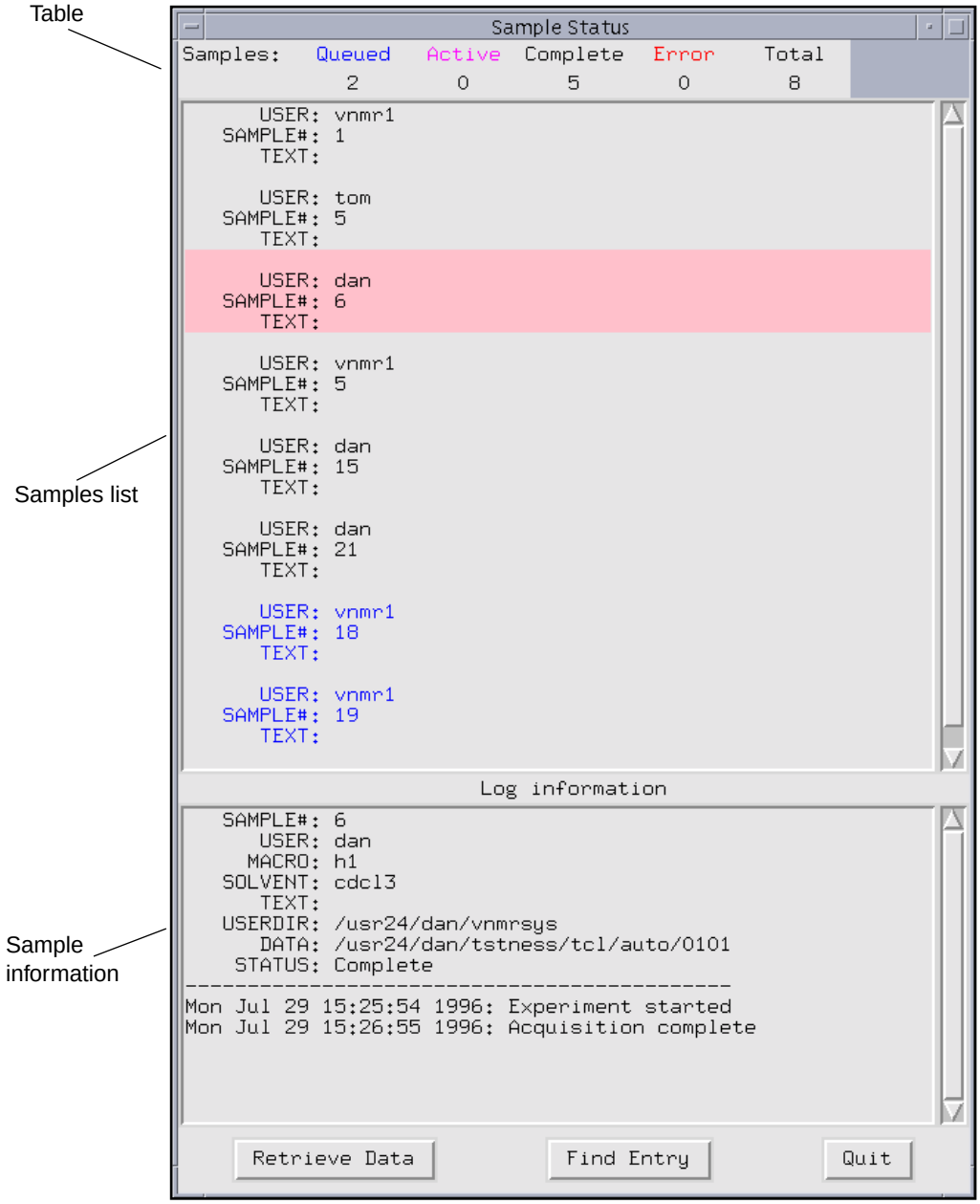


Figure 73. Sample Status Window (`status` Program)

each sample in the list below. For example, if the row in the table for queued samples is blue, each entry in the list below displayed in blue indicates a queued sample.

- To see detailed information of a particular entry, **click on the entry to highlight it**. The detailed information appears in the Log information section of the Status window.
- To retrieve the selected entry into the currently-joined experiment, click on **Retrieve Data**. This button is active only if data is present in the selected entry.

- To sort the entries or find a particular entry in the Status window, click on **Find Entry**. The locate window appears (see Figure 74).

  You can sort the list chronologically, by user, by location, or by status. Additionally, if you click on the label of one of the sort types, a pull-down menu is displayed so that you can further refine the sort. For example, if you click on the **by User** label, a list of user names appears. Select the user you want to put at the top of the list, and automatically the entries in the list change position according to your selection.

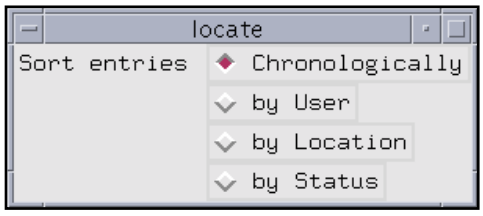


Figure 74. Locate Window (`status` Program)

Using Sample Changers in Continuous Walkup Mode

You can use a sample changer throughout the day in a continuous walkup mode. This mode enables you to add samples to the queue throughout the day.

1. Prepare your sample and place it in an available location in the carousel or in a sample tray.
2. Enter **`walkup`** in the VNMR input window.

   The Sample Entry Form window (see Figure 75) appears.

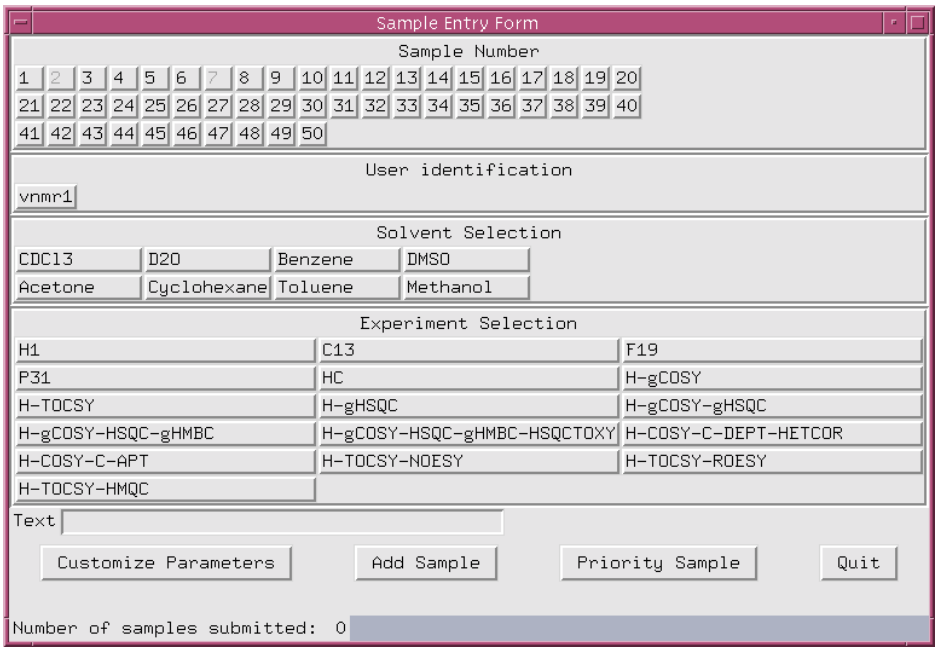
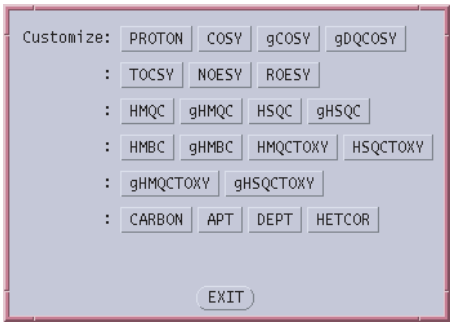

Sample Entry Form
Sample Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50
User identification
vnmr1
Solvent Selection
CDCl3 D2O Benzene DMSO
Acetone Cyclohexane Toluene Methanol
Experiment Selection
H1 C13 F19
P31 HC H-gCOSY
H-TOCSY H-gHSQC H-gCOSY-gHSQC
H-gCOSY-HSQC-gHMBC H-gCOSY-HSQC-gHMBC-HSQCTOXY H-COSY-C-DEPT-HETCOR
H-COSY-C-APT H-TOCSY-NOESY H-TOCSY-ROESY
H-TOCSY-HMQC
Text
Customize Parameters
Add Sample
Priority Sample
Quit
Number of samples submitted: 0

Figure 75. Sample Entry Form Window for Walkup Operation

3. Fill in the Sample Entry Form window.
   - **Sample Number** – select the location(s) to which you added samples. The sample locations that are grayed out are already in the day's queue.
   - **User Identification** – select the user.

- **Solvent Selection** – select the solvent for the chosen location(s).
- **Experiment Selection** – select one or more experiments for the chosen location(s).
- **Text** – enter information about the experiment, if desired.
- To customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles, click the **Customize Parameters** button.

  Click on the item you want to customize. A new window opens and provides appropriate parameters to change.



- When finished with this location (or locations), click **Add Sample** or **Priority Sample**.

  Add Sample – appends your requested samples and experiment to the current `enterQ` file.

  Priority Sample – puts your requested samples and experiment at the head of the `enterQ` file. You can configure this button to request a password.

  Quit – exits the program without adding samples to `enterQ`.

The `walkup` macro creates a new daily automation directory named `auto_dd.mm.yy`, where `dd` is the current day of the month, `mm` is the month, and `yy` is the year (e.g., `auto_01.04.99`). The automation directory for each day is saved in the directory specified by the global parameter `globalauto`. If the parameter and/or directory do not exist, the `walkup` macro creates them.

An alternative is to call the `walkup` macro from a menu button. A prototype `asm` menu with the buttons Walkup, Status, and Return has been put in `menulib`. The Status button is useful because it runs the status program to monitor the status of an automation run and it can retrieve data into VNMR for viewing. The `asm` menu can be made accessible from the Main menu by removing the comment symbols on the lines near the bottom of the `menulib/main` file.

The walkup macro also permits a fully continuous automation queue in which only the sample being run and any queued locations are inaccessible (grayed out). To enable this mode of operation, change the following line in the file `/vnmr/asm/auto.conf`:

From –

```
set exList {exp1/sampleinfo locQ}
```

To –

```
set exList {exp1/sampleinfo}
```

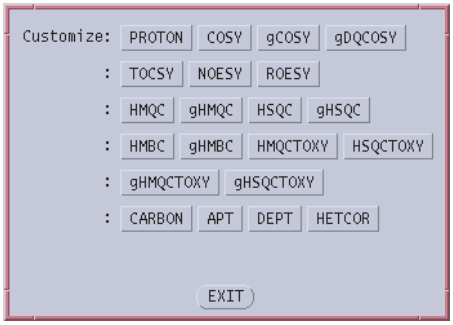
Adding Samples to an Automation Run in Progress

You can add samples or experiments to an automation run already in progress.

1. Prepare your sample and place it in an available location in the carousel or in a sample tray.
2. Type **`enter`** in the VNMR input window.

   A Sample Entry Form window appears, similar to Figure 75.

3. Fill in the Sample Entry Form window.
   - **Sample Number** – select the location(s) to which you added samples. The sample locations that are grayed out are already in the day's queue.
   - **User Identification** – select the user.
   - **Solvent Selection** – select the solvent for the chosen location(s).
   - **Experiment Selection** – select one or more experiments for the chosen location(s).
   - **Text** – enter information about the experiment, if desired.
   - **Customize Parameters** – Click on this button to customize any of the experimental parameters, including spectral width, number of scans, delays, and pulse angles. Click on the item you want to customize. A new window opens and provides appropriate parameters to change.



   - When finished with this location (or locations), click **Add Sample** or **Priority Sample**.

     Add Sample – appends your requested samples and experiment to the current `enterQ` file.

     Priority Sample – puts your requested samples and experiment at the head of the `enterQ` file. You can configure this button to request a password.
   - **Quit** – exits the program without adding samples to `enterQ`.

9.5 Changing Sample Changers or Serial Ports

To change to a different sample changer or to change to a different serial port, you must stop and restart `acqproc` by entering `su acqproc` twice:

```
>su acqproc
>su acqproc
```

9.6 Using Gradient Autoshimming with Automation

Gradient autoshimming requires the Automated Deuterium Gradient Shimming module to be installed.

Before you can run gradient shimming in automation, you must set up gradient shimming and map the shims with deuterium as described in the section on gradient autoshimming in the section "Deuterium Gradient Shimming," page 383.

After the shims are mapped, set `wshim='g'` in the parameter sets to be used with the automation run.

You might also wish to customize the macros `setlk` and `gmapz` for gradient shimming with different solvents. See "Example of Customizing a Macro," page 303, for more information.

9.7 Automation Run Description

Table 45 lists the commands and parameters related to automation run operation.

Table 45. Automation Mode Commands and Parameters

Commands	
`aa`	Abort acquisition with error
`auto<(automation_dir>`	Prepare for an automation run
`auto_au`	Controlling macro for automation
`autogo<(file<,automation_dir>)>`	Start automation run
`autogo<(file<,automation_dir>)>`	Start automation run
`autosa`	Suspend current automation run
`enter<(file<,<config_file>)>`	Enter sample information for automation run (VNMR)
`enter <file> <config_file>`	Enter sample information for automation run (UNIX)
`gilson`	Open the Gilson Control window
`halt`	Abort acquisition with no error
`status<(directory<config_dir>)>`	Display status of sample changer (VNMR)
`status directory <config_dir>`	Display status of sample changer (UNIX)
`walkup`	Walkup automation
Parameter	
`auto` {'y','n'}	Automation mode active
`autoname`	Prefix for automation data file
`gilpar` {array of 4 integers}	VAST sample changer values
`loc` {0, 1 to traymax}	Location of sample in tray
`saveglobal` {array of parameter names}	Save selected parameters from global tree

In an automation run, the user starts by using the `enter` program to provide information about the nature of up to 100 samples and the experiments to be performed on them.

After the information is entered and the automation run started, the user can run the `status` program to find out which experiment have been completed, which are in progress, and which are still in the queue. As part of an automation run, a single directory is created to handle the data collection. You can choose where the acquired data sets are to be distributed.

The `enter` program keeps track of which locations in the sample tray have been assigned, which are not assigned, and allows entry of information about each sample in a simple format. The `enter` program can be considered the “tray manager.” The `enter` program requires the user to input information about the sample and the experiments to be run on a particular sample. If a method is already defined for performing a typical experiment, the name of the macro that describes that method (for example, `h1` or `hc`) can be entered or selected, and that experiment or experiments will be run.

Sometimes, however, a particular sample requires other conditions for data acquisition; this is not a problem because `enter` also allows a complete line of keyboard entry to describe the experiment to be run; for example, `setup('H1','dmso') nt=4 pw=10` would be a legitimate entry. You can customize the selection of what information is requested and in what manner the information is requested. See the manual *VNMR User Programming* for details.

During the automation run, three files in the automation directory keep track of the status of different experiments:

- `enterQ` file holds the entries for the experiments waiting to be submitted.
- `psgQ` holds the experiment in the process of being submitted.

- `doneQ` file holds both active and completed experiments.

The `status` window automatically displays the contents of these files and enables the user to closely examine individual files. As the automation run proceeds, the `status` program updates its display to reflect the current status of the run.

An automation run uses its own experiment files, `exp1` through `exp4`, which reside in the automation directory; thus the "automation" experiments are separate and distinct from any experiments in the files of individual users. After data is accumulated, you can choose where the collected data is stored. Data can be saved in the following locations:

- In the automation directory
- In subdirectories of the automation directory
- At any arbitrary directory

Basic Automation Run

An automation run involves three basic steps:

1. Enter information about the samples to be run during the sample changer run.

   The `enter` program provides a mechanism to define essential information about the sample and the experiment to perform. Depending on the specific details of the `enter` program configuration, sample and experiment information might be selected by pressing a button or typing in information.

   The sample entry process might typically occur during the course of the day (assuming a common case of open access operation during the day, and sample changer run at night). One or more operators can use the `enter` program to create a file of information about the samples and experiments they wish to run.

   If multiple users are involved, the situation becomes slightly complicated because all the information must in the end reside in a single file. Either different users could enter information into a single file consecutively or they could enter information into different files simultaneously, with the system operator required to then merge these various files into a single file before the automation run is started. Since the file that is created is a simple text file, the latter task is easily accomplished with standard UNIX tools.

2. Create a directory for your "automation directory" to hold the various "queues," the automation experiments, and perhaps the data collected during the run.

   To do this, use the `auto` program. This can be done before or after step 1, or even automatically when the automation run is started.

3. Enter `autogo` to start the run.

   The `autogo` command starts the automation run, submitting the first experiment and holding subsequent experiments in a queue, ready to be submitted one at a time as the previous experiment terminates.

   Note that the experiment queueing facility is not used, and that only one experiment is typically submitted at a time. This fact makes it easier to change the order of experiments in the queue once the automation run is in progress, to add experiments to the queue, etc.

Sometimes at the start of an automation run, there is a sample already in the magnet. Often, the system does not know the tray location to which that sample should be returned. The result is that the sample is returned to position zero in the sample tray. The `sethw` command provides a convenient method of identifying the location the sample should be

returned to (e.g., `sethw('loc',22)` causes the sample to be returned to location 22 in the sample tray).

Automation Behind the Scenes

An automation run consists of two main items: a file containing information on the samples to be run and a directory containing the data along with supporting automation files. Samples are entered using the `enter` program, which is specifically designed for automatic sample entry. You should enter the proper information. Once the information is entered, it is saved as a simple text file named by you. If you examine this file, you will see that the last item of each entry, `Status`, is set to queued.

An automation run does not begin until the command `autogo` is issued. You can enter `autogo` at the command line in VNMR to initiate automation (see the *VNMR Command and Parameter Reference* for further details about `autogo`).

Several things happen when `autogo` is issued. First, a directory is created to hold the automation data. The directory path is contained in the VNMR global variable `autodir`. Next, several subdirectories and files are created within this main data directory. Automation creates a new set of experiments (`exp1` to `exp4`) to temporarily hold the data during acquisition. The experiments reside in `$autodir`. Other critical files created are `enterQ`, `doneQ`, `psgQ`, `locQ`, and `sampleinfo`.

To examine what the system is doing during an automation run, open a console window. This window echoes each event as it happens. Experiments to be performed are copied by `autogo` from the file created by the `enter` program to the `enterQ` file. As each sample is submitted for acquisition, the information about the sample is removed from the `enterQ` file and placed in the `sampleinfo` file. After submission, the information is written into the `doneQ` file.

The `psgQ` file holds information generated by a pulse sequence, e.g., S2PUL. `psgQ` is used by the automation system to start an acquisition.

The `locQ` file contains `loc` values that have been used during the automation run. This file is created by the `auto_au` macro. During walkup operation, the `enter` program uses the `locQ` file to determine which sample locations are available for another user.

The `sampleinfo` file contains information on the sample currently being run. This file is accessed by the acquisition macros to retrieve needed information. The `sampleinfo` file is placed in the appropriate experiment directory. The `doneQ` file contains information about *both* active and complete experiments.

The `enterQ` file is the critical file for sample management. Once an automation run is started, the only way to add more samples, delete samples, and so forth, is to directly modify the `enterQ` file. `enterQ` can be updated manually according to the procedures given later in this chapter. When the `enterQ` file contains no entries, the system recognizes that automation is complete.

While an Automation Run is in Progress

Automation mode is a separate mode of operation from the normal multiexperiment mode and must be kept distinct from it. An automation run can only be initiated when all other acquisitions are finished. Likewise, all data acquisition of an automation run must be finished (or at least paused) before the system can be returned to the multiexperiment mode of data acquisition. You should realize that these statements apply only to acquisition. Data processing is possible using any experiment. Data can be recalled, processed, and plotted

in the normal way. Because the automation run acts in effect as a separate user, you will have no problems trying to plot at the same time as the automation run.

Also, by using the `status` command (see Figure 73), data that is part of the automation run and already finished (including 2D runs that are still in progress but for which some of the FIDs have already been acquired) can be recalled using the mouse and then processed or reprocessed. Of course, any processing that has been specified to take place as part of the automation run will occur at the appropriate time separately and concurrently with any processing you may be doing.

Interacting with the acquisition itself during an automation run is more limited than in multiexperiment mode. You cannot start an acquisition, but can abort the current acquisition using the Abort Acquisition button or by issuing the `aa` command. Depending on what you have used for the `werr` parameter, it is likely that an abort of an acquisition in progress terminates the acquisition with no processing whatsoever. Instead of entering `aa`, you can enter `halt`, in which case the normal end of experiment (`wexp`) processing occurs.

As the automation run progresses, experiments are removed from the `enterQ` file, placed the `sampleinfo` file, and then placed into the `doneQ` file as they are active or completed. All processing messages that occur during the automation run appear in the console window, and thus the progress of the automation run can be easily monitored by leaving the console window open. The `status` command can be used to monitor this progression.

When an Automation Run is Finished

At the finish of an automation run, the system automatically returns to the “normal” mode and normal data acquisition can be initiated with the `go` or `au` commands.

If you reach the end of the automation run and wish to continue the run with more samples, use the `enter` program to enter new samples into a new sample file (or perhaps you already did this while the automation run was in progress). Then enter `autora`.

The `autoname` parameter controls the file names to be used and can use the value of VNMR parameters as part of the file name. For a complete description of the `autoname` parameter, see the *Command and Parameter Reference* manual.

Parameters for Automation

Basic parameters for automation are recalled from the directory `stdpar`. This directory can be owned by the user who started `autogo` or it can reside in the `/vnmr` directory, in which case the directory is not alterable by anyone other than `vnmr1`.

`stdpar` should contain an entry for each nucleus. For example, the `stdpar/H1.par` file is retrieved by the macro `h1` and used to run the proton NMR spectrum. To a significant extent, acquisition, processing, and plotting can be controlled by parameters preset in `stdpar`. Some parameters in `stdpar` that directly affect functioning of automation (not including general parameters such as `pw`) are listed in Table 46 with descriptions of values.

Certain global parameters are also important for the correct submission of additional acquisitions. The `saveglobal` global parameter saves an array of these global parameter names. Whenever a `go`, `ga`, or `au` is entered, the parameters listed in the array are copied to the current experiment parameter tree, and an underscore is appended to the parameter names (e.g., `loc` becomes `loc_`). Some of the global parameters saved are `loc`, `lockpower`, `lockphase`, `lockgain`, `z0`, `lkof`, `gilpar`, `pkpick`, and `parstyle`. Additional global parameter names can be added to the `saveglobal` array.

Table 46. Basic Parameters for Automation

Parameter	*Value*	*Description*
alock	's'	Autolock only on sample insert.
	'y'	Lock using parameters based on the solvent. Lock phase and lock gain are not adjusted (quick lock mode).
	'u'	Do not lock (does not apply to *MERCURY* or *GEMINI 2000*).
	'a'	Autolock searches for lock resonance and adjusts parameters lockpower and lockgain.
wshim	's'	Shim using shim method specified by the parameter method.
	'g'	Automatic shimming using gradient shimming is done only at beginning of the first experiment, following the sample change. method is ignored. This value is available only in automation; it is not used with go, ga, or au.
method	'z1z2'	Shim gradients Z1 and Z2 with a criterion of medium to medium, total time 60 seconds or less (default method).
gain	'n'	System adjusts gain before acquisition.
spin	'n'	System does not regulate spinning before acquisition.
	20	System tries to adjust spin speed to 20 Hz before starting acquisition. If speed does not regulate and in='y', ejects the sample and proceeds to the next sample. If in='w', only a warning message is added to the log file and acquisition continues with the unregulated speed.
intmod	'off'	No integrals are plotted (typical for c13 parameter set).
	'partial'	Integrals are plotted for each peak separately (typical for h1 parameter set). The region command determines integrals.
pltmod	'off'	Suppress plotting. If an error occurs when processing (e.g., aph failure), the processing macro sets pltmod to off and no plotting occurs.
	'fixed'	Plot spectrum using stdpar parameters. This allows setting all plotting-related parameters when stdpar is saved.

The saveglobal parameter is also saved as saveglobal_, even though it is not an explicit member of the array.

Whenever any conditional processing occurs, the saveglobal_ list of parameters is copied back into the global tree. By doing this, the processing macros have access to the values used to initiate the acquisition. Whenever a go, ga, or au is executed, any parameters listed in the experiment's saveglobal_ parameter are deleted before the new parameters from the global saveglobal parameter are copied.

Variable Temperature Control During Automation

Variable temperature operation during an automation run proceeds in a straightforward way. The sequence of events is the following:

1. The current sample is removed from the magnet by the sample changer.
2. The new temperature is set and the temperature starts to change, at a rate of no more than 12 degrees per minute.
3. When the new temperature is reached, the system checks if the tin parameter is set to 'y'. If so, the system waits for the temperature controller to achieve regulation.

The VNMR parameter `vtwait` determines how long the system waits for the VT to regulate. The default is 300 seconds (5 minutes). If you are making a large temperature change, or have the VT controller slow rate set to very slow, you might need to change this default. If the VT does not regulate before this time and `tin='y'`, the system will move to the next sample.

4. The sample changer inserts the sample into the magnet.
5. If spinning is requested, the sample spin regulation system starts.
6. If a `pad` delay is requested, that delay occurs. This delay allows the sample temperature to come to equilibrium.
7. The sequence then proceeds in the normal fashion, doing locking, shimming, and receiver gain adjustment, as requested.

9.8 Customizing the Sample Entry Window

The choices displayed by the `enter` program can be customized. The *users*, *solvents*, and *experiments* are specified in text files in the `/vnmr/asm` directory. Each text file is specified as pairs of lines of entries. The first line of each pair is the label that will be displayed in the Sample Entry Form window by the `enter` program. The second line is what will be written into the text file generated by the `enter` program when that particular labeled item is selected.

Listing 3 shows the default contents of the `experiments` text file. The `solvents` file and `user` file are similarly constructed.

9.9 Automated Data Acquisition

As previously stated, when entering information about the sample, experiments are most easily begun by selecting the name of a macro from the `enter` program. Any user-created macro, with associated arguments (if any), can also be selected by the `enter` program, so that any particular experiment needed can be specifically programmed. A complete description of automation macros appears in the *VNMR Command and Parameter Reference*.

In the `enter` program, these macros are typically used as a simple name and without any of the optional arguments that may be used if the macro is used “by itself.” The solvent, which is required by each of these macros when run in the multi-experiment mode, is automatically added by the `enter` program.

Note that multiple entries in the `enter` file may refer to the same sample. Thus it is not necessary to use a “combined” experiment like `hc` to run a proton and carbon experiment on a particular sample. Instead, you can fill out one entry with `h1` and then a second for the same sample with `c13`.

All of the standard single-experiment automation macros set the `wexp` parameter to `'procplot'`. All of the standard combined-experiment automation macros set the `wexp` parameter to `'autolist'`. None of the standard automation macros include a call to `au` (this is a change from earlier operation of these macros because they used to include the call to `au`). Therefore, customization in the `enter` program is possible by entering the standard macro followed by the changes (e.g., `h1 nt=4` selects the standard `h1` experiment but runs it with four transients).

Listing 3. Contents of Default `/vnmr/asm/experiments` File

```
H1
AuHexp
C13
AuCexp
F19
AuF
P31
AuP
HC
AuHexp(solvent,`CARBON`)
H-gCOSY
AuHexp(solvent,`gCOSY`)
H-TOCSY
AuHexp(solvent,`TOCSY`)
H-gHSQC
AuHexp(solvent,`gHSQC`)
H-gCOSY-gHSQC
AuHexp(solvent,`gCOSY`,`gHSQC`)
H-gCOSY-HSQC-gHMBC
AuHexp(solvent,`gCOSY`,`HSQC`,`gHMBC`)
H-gCOSY-HSQC-gHMBC-HSQCTOXY
AuHexp(solvent,`gCOSY`,`HSQC`,`gHMBC`,`HSQCTOXY`)
H-COSY-C-DEPT-HETCOR
AuHexp(solvent,`COSY`,`CARBON`,`DEPT`,`HETCOR`)
H-COSY-C-APT
AuHexp(solvent,`COSY`,`CARBON`,`APT`)
H-TOCSY-NOESY
AuHexp(solvent,`TOCSY`,`NOESY`)
H-TOCSY-ROESY
AuHexp(solvent,`TOCSY`,`ROESY`)
H-TOCSY-HMQC
AuHexp(solvent,`TOCSY`,`HMQC`)
```

During an automation run, the experiment information from the `enterQ` file is placed in `exp1` and in the `autodir` directory. The `auto_au` macro is then called. This macro reads the `sampleinfo` file and sets the `solvent` and `loc` parameters, fills in the text, and executes the command defined by MACRO. After that, `auto_au` examines the value of the `wexp` parameter. If `wexp` is set to `'procplot'`, then `auto_au` calls `au`. If `wexp` is set to `'autolist'`, then `auto_au` inserts `'auto'` as the first argument to `autolist` and calls `au('wait')`. If `wexp` is set to anything else, `auto_au` does not call `au`. As a result, any existing automation macros with a built-in call to `au` will still work as long as `wexp` is not set to `'procplot'` or `'autolist'`.

When `au` is executed, the global parameters listed in the `saveglobal` parameter are copied into the experiment parameter list. Each parameter name is appended with an underscore (_). Whenever any conditional processing occurs (e.g., `wexp`), the previously saved parameters are returned to the global tree. The conditional processing macros therefore have access to the correct values of global parameters, such as `loc` and the lock parameters.

Optimizing Acquisition Macros

Acquisition in automation is usually initiated by macros such as `h1` and `c13`. By understanding the order of events in acquisition, automation can be optimized.

The macro first retrieves the appropriate `stdpar` file. Important parameters such as `pw90` and `tpwr` are retrieved from the `probes` file. The `.def` files determine default `sw`, `nt`, etc. In addition, the parameters that directly affect automation (`alock`, `wshim`, etc.) should be set appropriately. These parameters are listed in Table 46 with explanations of reasonable values. Once the `stdpar` file has been retrieved, the macro calls `setlk`. After calling `setlk`, acquisition begins.

Listing 4 is the text of the `AuHexp` macro.

Listing 4. Contents of `AuHexp` Macro

```
if ($# > 0) then $solv=$1 else $solv=solvent endif

setup('H1',$solv)
Autoclrexp
wexp=''
Autosetgpar
if (auto <> 'y') then
  explist='PROTON','glidewexp'
else
  explist='PROTON' setlk(solvent)
endif

if ($# > 1) then
  $arg=''
  $x = 2
  repeat
    format($x,0,0):$arg
    if typeof('$'+$arg) then
      AutoAddEXP(${$x},'nodialog')
    endif
    $x=$x+1
  until $x > $#
endif

macro=$0 pw(45) seqfil='s2pul'
nt=8 clear(2) d1=1 wbs='' wnt=''
setsw(14,-2)
Automacrodir('make')
Autosetwexp
dg
```

Data is processed by the macro `procplot`, which is called by `autolist`. The `procplot` macro processes and plots both 1D and 2D data. A full explanation of `procplot` and associated macros is provided in the *VNMR Command and Parameter Reference.*

Customizing Macro Operation

Extensive opportunity exists for customizing the operation of existing macros—this customization can always occur on either the systemwide level (in the `/vnmr` directory) or

at the user level (in the user's personal `vnmrsys` directory). The starting parameter sets can be modified to contain parameters of your choosing. And finally, the macros themselves (`h1`, `c13`, `setlk`, etc.) can be modified. An obvious recommendation is to make such changes at the single-user level first, and then determine their acceptability before making the changes at the system-wide level.

Other possibilities for customizing include macros used during an automation run:

- `h1p`, `c13p`, `f19p`, and `p31p` for processing.
- `hregions` to select integral regions in a proton spectrum.
- `react` for error processing.
- `getsn` to get a signal-to-noise estimate and `testsn` (which uses the `testct` macro and the `sn` parameter) for signal-to-noise checking.
- `cleanexp` to remove old experiment files and directories.
- `cptmp` to copy experiment data into an experiment subfile.
- `setlk` for setting up automatic locking choices (see example in next section).

Table 47 lists commands and parameters related to customizing macro operation.

Table 47. Commands and Parameters for Customizing Macro Operation

Commands	
`au*`	Submit experiment to acquisition and process data
`auto_au`	Controlling macro for automation
`c13p`	Process 1D carbon spectra
`cleanexp<(file1<,file2,...>)>`	Remove old files and directories from an experiment
`cptmp<(file)>`	Copy experiment data into experiment subfile
`f19`	Process 1D fluorine spectra
`fixup`	Adjust parameter values selected by setup macros
`getsn:current_sn,predicted_sn`	Get signal-to-noise estimate of a spectrum
`h1p`	Process 1D proton spectra
`halt`	Abort acquisition with no error
`hregions`	Select integral regions in proton spectrum
`p31p`	Process 1D phosphorus spectra
`react<('wait')>`	Recover from error conditions during werr processing
`resume`	Resume paused acquisition queue
`setlk(solvent)`	Set up lock parameters
`testct`	Check ct for resuming signal-to-noise testing
`testsn`	Test signal-to-noise of a spectrum
`* au<(<'nocheck' ><,><'next'><,><'wait'>)>`	
Parameters	
`auto` {'y','n'}	Automation mode active
`autodir` {string}	Automation directory absolute path
`autoname` {string}	Prefix for automation data file
`lastlk` {string}	Last lock solvent used
`sn` {number}	Signal-to-noise ratio

A number of commands (`au`, `halt`, `resume`, etc.) are important in constructing macros for sample changer operation and are more or less unique to that mode in that they are rarely if ever used at other times. Note that `jexp` cannot be used in an automation macro. A number of parameters are also important in constructing macros for sample changer operation. These parameters are rarely if ever used at other times.

Example of Customizing a Macro

The macro `setlk` can be customized for each system to enhance autolocking and autoshimming. Because many of these enhancements are system specific, various lines in `setlk` are commented out and the system macro is nonfunctional until modified by the system administrator. As a result, it is advisable to explicitly set the values for the `z0`, `lockpower`, and `lockgain` parameters as discussed in item 3 below. Listing 5 provides an example of a modified `setlk` macro.

For each sample, `setlk` permits the system to perform the following adjustments:

1. Retrieve solvent-based shim sets. Starting from a shim set for each solvent has proven useful on many automation systems. To retrieve a set of shims for each solvent, remove the quotes around the following line in `setlk`:
   ```
   rts($1):$e
   ```
   Alternatively, separate shims for each solvent may not be necessary but specific shims for only one or two solvents may be desirable. The following statements show how to implement this scheme:
   ```
   format($1,'lower'):$solv
   if ($solv='d2o')
     then rts('d2o')
     else rts('cdcl3')
   endif
   ```
   In this example, if the solvent is `d2o`, then specific shims for `d2o` are called; otherwise, `cdcl3` shims are used as a starting point for all organic solvents.

2. Choose a specific shim method based upon the T_1 of the individual solvents. Quotes should be removed from the following lines in `setlk`:
   ```
   if ($solv='acetone')or($solv='cd3od')or($solv='cd2cl2')
     then method='longt1'
     else if ($solv='c6d6')or($solv='cdcl3')
       then method='medt1'
       else if ($solv='dmso')or($solv='d2o')
         then method='shrtt1'
         else method='zall'
       endif
     endif
   endif
   ```

3. Explicitly set `z0`, `lockpower`, and `gain`. This is a useful technique to improve the autolock reliability. This is especially apparent for the first sample of an automation run. If the lock power has been low, this first sample may not be run because of an autolock failure.

   To use this feature effectively, lock and shim on a sample in each one of the solvents that you will normally use. Record the values of `z0`, `lockpower`, and `gain` that produce a stable lock.

   Remove the quotes from the following lines in `setlk` and input the appropriate values of `z0`, `lockpower`, and `gain`. Notice that the example below shows some of the entries with the `z0` values inserted. The number represents the value of `z0` as shown by the lock display in `acqi`. Replace the numbers with the appropriate values for your system.
   ```
   if $solv='cdcl3'
     then Z0=128 lockpower=37 lockgain=41
     else if $solv='d2o'
       then Z0=35lockpower=37 lockgain=48
   ```

```
            else if $solv='acetone
              then Z0=12 lockpower=21 lockgain=39
              else if $solv='dmso'
                then lockpower=33 lockgain=36
                else if $solv='c6d6'
                  then lockpower=20 lockgain=39
                  else if $solv='cd3od'
                    then lockpower=25 lockgain=35
                    else lockpower=30 lockgain=40
                  endif
                endif
              endif
            endif
          endif
```

In addition, the macro `gmapz` can be customized to run gradient shimming on different solvents. Uncomment the appropriate lines in the following section of the macro:

```
"uncomment this section for automation (wshim='g')"
  if (auto='y' and tn='lk') then
    $solv='' format(solvent,'lower'):$solv
"add other solvents with long T1"
    if ($solv='acetone') then
      d1=d1*2
    endif
"add other solvents with weak signal"
    if ($solv='cdcl3') then
      nt=nt*4
    endif
  endif
```

Listing 5 shows a more extensively customized `setlk` macro that handles multiple probes and provides solvent-based parameter sets, all based upon the system probe files.

Listing 5. Modified `setlk` Macro

```
exists('probe','parameter','global'):$ep
if ($ep > 0.5) and (probe <> '') then
  $ep = $ep
else
  $ep = 0
endif
if ($# < 1) then
  $solv = solvent
else
  $solv = $1
endif
"if solvent-based shim sets are desired for new solvents,store"
"appropriate shim sets (if you use more than one probe you should "
"have a shims library for each) then remove quotes from the next line "
format($solv,'lower'):$solv
if $solv = 'benzene' then $solv = 'c6d6' endif
```

Listing 5. Modified `setlk` Macro (continued)

```
if ($ep) then  "probe exists & has a value "
  $shimfile = userdir+'/shims/'+probe+'/'+$solv
  exists($shimfile,'file'):$eshims
  if ($eshims > 0.5) then
    rts($shimfile)
  else
    $shimfile = '/vnmr/shims/'+probe+'/'+$solv
    exists($shimfile,'file'):$eshims
    if ($eshims > 0.5) then
      rts($shimfile)

    else
      write('error','Sorry, no shims for this probe and solvent')
    endif
  endif
else
  rts($solv)  "This happens if no probe-specific shim directories"
endif
"if shim method is to be determined by solvent, then remove the"
" quotes in next lines (you must create the methods given)"
if
($solv='acetone')or($solv='cd3od')or($solv='cd2cl2')or($solv='cd3cn')
  then method='longt1'
else if ($solv='c6d6')or($solv='cdcl3')or($solv='thf')
  then method='medt1'
else if ($solv='dmso')or($solv='d2o')
  then method='shrtt1'
else method='zall' endif endif endif
"if you prefer to explicitly set lockpower/lockgain for each solvent,"
"the lines below must be updated to correspond to"
"the non-saturating values for the spectrometer in use. this"
"can speed up the automatic locking process."
"z0 can also be set (for weak locks), along with lockpower/lockgain"
if ($ep > 0.5) then
  if probe = 'asw305' then
    if $solv='cdcl3'   then z0=415 lockpower=25 lockgain=32  else
    if $solv='d2o'     then z0=536 lockpower=15 lockgain=29  else
    if $solv='acetone' then z0=630 lockpower=12 lockgain=24  else
    if $solv='dmso'    then z0=629 lockpower=20 lockgain=26  else
    if $solv='c6d6'    then z0=420 lockpower=20 lockgain=24  else
    if $solv='cd3od'   then z0=575 lockpower=24 lockgain=26  else
    lockpower=20 lockgain=25 endif endif endif endif endif endif
  else if probe = 'pfgid' then
    if $solv='cdcl3'   then z0=413 lockpower=14 lockgain=29  else
    if $solv='d2o'     then z0=534 lockpower=12 lockgain=24  else
    if $solv='acetone' then z0=628 lockpower=6 lockgain=19  else
    if $solv='dmso'    then z0=630 lockpower=11 lockgain=24  else
    if $solv='c6d6'    then z0=416 lockpower=10 lockgain=20  else
    if $solv='cd3od'   then z0=573 lockpower=24 lockgain=24  else
    lockpower=24 lockgain=40 endif endif endif endif endif endif
```

Listing 5. Modified `setlk` Macro (continued)

```
  else if probe = 'sw231' then
    "values in this section are for the AutoSW probe "
    if $solv='cdcl3'   then z0=452 lockpower=26 lockgain=29  else
    if $solv='d2o'     then z0=592 lockpower=15 lockgain=29  else
    if $solv='acetone' then z0=702 lockpower=12 lockgain=20  else
    if $solv='dmso'    then z0=690 lockpower=20 lockgain=26  else
    if $solv='c6d6'    then z0=452 lockpower=20 lockgain=24  else
    if $solv='cd3od'   then z0=630 lockpower=24 lockgain=20  else
    lockpower=20 lockgain=25 endif endif endif endif endif endif

  else if probe = '10mm' then
    if $solv='cdcl3'   then z0=414 lockpower=26 lockgain=46  else
    if $solv='d2o'     then z0=533 lockpower=22 lockgain=42  else
    if $solv='acetone' then z0=696 lockpower=16 lockgain=30  else
    if $solv='dmso'    then z0=603 lockpower=20 lockgain=42  else
    if $solv='c6d6'    then z0=415 lockpower=20 lockgain=40  else
    if $solv='cd3od'   then z0=570 lockpower=24 lockgain=40  else
    lockpower=24 lockgain=40 endif endif endif endif endif endif
  endif endif endif endif "close probe-specific section"
else "probe variable does not exist or is not set"
  "values currently in this section are for AutoSW probe "
  if $solv='cdcl3'   then z0=415 lockpower=26 lockgain=29  else
  if $solv='d2o'     then z0=539 lockpower=15 lockgain=29  else
  if $solv='acetone' then z0=629 lockpower=10 lockgain=20  else
  if $solv='dmso'    then z0=631 lockpower=20 lockgain=26  else
  if $solv='c6d6'    then z0=415 lockpower=20 lockgain=24  else
  if $solv='cd3od'   then z0=570 lockpower=24 lockgain=20  else
  lockpower=20 lockgain=25 endif endif endif endif endif endif
endif "end of z0/power/gain section"

  " alock = 'y' "
```

9.10 Automated Data Processing

In an automation run, any processing done as part of the experiment is governed by four parameters: `werr`, `wbs`, `wnt`, and `wexp`.

`werr` contains the name of the macro that runs if an error is detected during the experiment (receiver overflow, lost lock, etc.).

- `wbs` contains the name of the macro that runs after each block of the acquisition, assuming that no other processing is occurring. `wbs` processing is not done, for example, if data processing is still going on for a previous sample. In automation mode, the basic use of `wbs` processing is for experiments in which it is desired to test the signal-to-noise ratio periodically in order the stop the experiment at a point when the desired ratio has been reached (discussed under the descriptions of `c13` and `testsn` in the *VNMR Command and Parameter Reference*).
- `wnt` applies to processing that must be done after each FID of a multi-FID experiment; therefore, `wnt` processing is rarely used in automation mode.

- `wexp` normally specifies a processing macro that performs data processing appropriate to the particular experiment that has been performed. This processing may include setting up and starting further experiments, based on the results of the first experiment. The most important processing to be done on each sample is selected by the `wexp` parameter.

If all four of these parameters are set to the null string (`wexp=''`), no data processing whatsoever is done, and the experiment is simply acquired and stored. Normally, however, you will want automatic data processing.

Whenever `wbs`, `wnt`, `wexp`, or `werr` processing occurs, the acquisition condition that initiated the processing is available from the parameter `acqstatus`. Many of the codes are related to sample changer operation. Refer to the manual *Getting Started* for more information about using acquisition codes. The codes are listed in the description of `acqstatus` in the *VNMR Command and Parameter Reference*.

9.11 File Structures in an Automation Run

This section describes the files and directories created in an automation run.

The command `enter('abc')` creates a directory named `abc` (referred to as the *automation setup directory*). The `abc` directory contains a file named `abc`, which lists experiment information, as follows:

```
varian> cat /export/home/vnmr1/abc
  SAMPLE#:   2
     USER:   vnmr1
    MACRO:   h1
  SOLVENT:   acetone
     TEXT:   ethyl-vanillin
  USERDIR:   /export/home/vnmr1/vnmrsys
     DATA:
   STATUS:   Queued
----------------------------------------
  SAMPLE#:   1
     USER:   vnmr1
    MACRO:   c13
  SOLVENT:   cdcl3
     TEXT:   menthol
  USERDIR:   /export/home/vnmr1/vnmrsys
     DATA:
   STATUS:   Queued
----------------------------------------
```

Also in the automation directory (in this case, `abc`) is a directory named `abc.macdir`, which contains *GLIDE*-related information for an automation run, including the following files:

```
varian> ls /export/home/vnmr1/abc/abc.macdir
curloc     loc1_AuHexp    loc2_AuCexp
```

For the automation run, the `autogo` command uses the information from the *automation setup directory* created by the `enter` program (`abc` in our example) and places information about current and completed experiments in a new directory, called the *automation directory*. For example, if `autogo('abc','abc1')` is entered, information is copied from the `abc` directory into the `abc1` directory created by `autogo`.

```
varian> ls /export/home/vnmr1/abc1
```

```
autoinfo  enterQ   enterQ.macdir  doneQ  exp1  exp2
exp3      exp4     psgQ           sampleinfo
```

The experiment files (`exp1`, `exp2`, etc.) are used for data acquisition and data processing. The `enterQ`, `psgQ`, `doneQ`, and `sampleinfo` files contain information about the experiments to be run. The `autoinfo` file is currently unused. The information from the file (e.g., `abc`) in the automation setup directory is copied to `enterQ`. As each sample is submitted for acquisition, the information about that sample is copied to `sampleinfo`. After the experiment is finished, the information about completed sample is moved from `sampleinfo` to `doneQ`. Information generated by pulse sequences is stored in `psgQ`.

A `sampleinfo` file typically looks like the following, containing information about the sample that is just about to be run, for possible use by the macros that are being executed:

```
varian> cat /export/home/vnmr1/abc1/exp1/sampleinfo
  SAMPLE#:   1
     USER:   vnmr1
    MACRO:   c13
  SOLVENT:   cdcl3
     TEXT:   menthol
  USERDIR:   /export/home/vnmr1/vnmrsys
     DATA:
   STATUS:   Queued
-----------------------------------------
```

The `sampleinfo` file is placed in the appropriate experiment directory and is saved with the data. After the automation run has run for a while, it might look like this:

```
varian> cd /home/vnmr1; ls abc1
0101.fid   autoinfo   enterQ   exp2   exp4
0201.fid    doneQ       exp1      exp3   psgQ   lab0401.fid
```

The new files, all containing the `fid` extension, are the data files collected during the acquisition run. Normally, as data is collected, the FIDs are placed into the automation directory with the name `xxyy.fid`, where `xx` is the sample location number and `yy` is an "experiment number" on that particular sample (`1` through `n`, where `n` experiments have been run on the same sample). If the user who started `autogo` sets the parameter `autoname`, then `autoname` is interpreted to determine the directory and file name where the data will be stored. The `autoname` parameter controls the version number attached to the file name and uses the value of VNMR parameters as part of the file name.

The data files have the following internal structure:

```
varian> cd abc1; ls 0101.fid
fid    log    procpar    sampleinfo   text
```

Of these files, a key file that relates to automation is the `log` file, which maintains a log of everything that happens during the run, for you to examine at a later time.

```
varian> cat 0101.fid/log
========> Wed Dec 25 17:05:18 1996 <==== New Exper: '0101'
17:05:17    Experiment Submitted.
17:05:18     Experiment Started ID = 1
   FID       Time      Error
   ---       ----      ---------------------------
17:09:06    Experiment Completed ID = 1
```

The `doneQ` file might look like this at this time:

```
varian> cat doneQ
   SAMPLE#:   2
      USER:   vnmr1
     MACRO:   h1
```

```
   SOLVENT:   acetone
      TEXT:   ethyl-vanillin
   USERDIR:   /home/vnmr1/vnmrsys
      DATA:   /home/vnmr1/slptest/0201
    STATUS:   Complete
----------------------------------------
   SAMPLE#:   1
      USER:   vnmr1
     MACRO:   c13
   SOLVENT:   cdcl3
      TEXT:   menthol
   USERDIR:   /home/vnmr1/vnmrsys
      DATA:   /home/vnmr1/slptest/0101
    STATUS:   Error
----------------------------------------
```

Another key file is `/vnmr/acqqueue/acquisitioninfo`, which contains information about the state of the acquisition computer after the last sample. This file is a text file that might look like this:

```
varian> cat /vnmr/acqqueue/acquisitioninfo
nt 16 ct 16 scale 0 np = 29952
gain 36 lockphase 256 lockpower 22 lockgain 53
```

Finally, the file `/vnmr/acqqueue/lastlk` contains information about the last lock solvent:

```
varian> cat /vnmr/acqqueue/lastlk
last lock solvent was cdcl3
```

Chapter 10. VAST Accessory Operation

Sections in this chapter:

- 10.1 “Using the VAST Accessory,” page 311
- 10.2 “Solvent Suppression in VAST,” page 317
- 10.3 “Processing, Displaying, and Plotting VAST Data Sets,” page 323
- 10.4 “Using CombiPlate to Analyze Data,” page 331
- 10.5 “Vast Process, Display, and Plot Macros,” page 337
- 10.6 “Preparing the Hardware and Configuring VNMR,” page 341
- 10.7 “Calibrating Volumes and Flow Rates,” page 345
- 10.8 “Acquiring Data on Standard Test Samples,” page 354
- 10.9 “Evaluating Carryover,” page 355
- 10.10 “VAST Interface Description,” page 355
- 10.11 “Customizing the enter Window for VAST,” page 362
- 10.12 “Files that Control VAST Operation,” page 363
- 10.13 “Writing VAST Protocols,” page 363

The VAST (versatile automatic sample transport) accessory uses the VNMR automation capability of UNITY*INOVA* and *MERCURY-VX* NMR spectrometers to take a sample from a vial or well on the Liquid Handler (see Figure 76) and move it into the Microflow probe. When the sample is in place, NMR data can be acquired. When the NMR experiment is finished, the sample is removed and either discarded into a waste container or returned to the source container. After removing the sample, the liquid path is then washed to minimize cross-contamination or carryover between samples.

The VAST accessory comprises the following:

- Gilson 215 Liquid Handler, Rheodyne (Gilson 819) injector and Valco valves
- VAST software interface to VNMR (sold separately, requires 6.1B or later)
- PEEK tubing, PEEK connectors, and signal cables
- Varian Microflow probe (sold separately)

10.1 Using the VAST Accessory

The VAST accessory is highly mobile and can be easily moved between spectrometers or moved away from the spectrometer when not in use. Therefore, a certain amount of setup might be required each time the VAST accessory is to be used.

After setting up, most of your interaction with the VAST autosampler will occur with normal VNMR automation controls, such as the `enter` program and with the `loc` parameter in the VNMR input window.

Figure 76. Gilson 215 Liquid Handler

To Prepare VAST for Use

1. Make sure the VAST accessory hardware is set up and that VNMR is configured, as described in "Preparing the Hardware and Configuring VNMR," page 341.
2. Calibrate the Z position of the arm (if necessary). Z position calibration is described in "Calibrations," page 361.
3. Make sure an appropriate waste container is properly installed on the rinsing station.
4. Prepare an appropriate solvent and make sure the inlet line to the syringe assembly is inserted into your solvent reservoir. Be sure to have enough solvent to complete the automation run.

CAUTION: **Make sure the sample/solvent you are using is compatible with, and does not react with, the sample container. For example, chloroform solvents will rapidly dissolve polystyrene microtiter plates. Microtiter plates can be made of polystyrene, polypropylene, polycarbonate, glass, and many other materials.**

5. In the VNMR input window, enter **`gilson`** to open the Liquid Handler window. Check the default settings in the Liquid Handler window. The default values are listed in Table 48.
6. Click the **Rack Def.** tab and configure the window for the racks to be used.
7. Click on the **Main Control** tab and then click on **Return Home**.

   This may take a few minutes. To speed up the process, press the STOP button on the front of the Liquid Handler, lift the needle, and slide the arm toward the left.
8. Prime the pump by clicking on the **Prime pump** button in the **Main Control** pane.

   The needle should automatically move over to the rinse station before the priming starts. Make sure to prime until you no longer see bubbles in the inlet line to the syringe module.

   The priming routine runs for about 3 minutes and uses the solvent attached to the inlet line.
9. Clear the NMR probe of any leftover sample or solvent by setting **Air valve** to **ON**.

   Let this run for about two minutes or until you hear air hissing out of the inject port. Be prepared to wipe up any sample or solvent that seeps out of the inject port.

Table 48. Typical Default Values in Liquid Handler Windows

Field	*Default Value**
SAMPLE Def. pane (Figure 104)	
Probe Volume	400
Sample Volume	350
Push Volume	50
Keep Sample	no
Number of Rinses	0
Rinse Delta Vol	10.0
Sample Well Rate	4.0
Sample Height	20
Sample Depth	NOSEEK
Needle Rinse Volume	1000 (for a 1000 µL syringe)
Needle Rinse Rate	16
Probe Slow Vol	100
Probe Slow Rate	0.3
Probe Fast Rate	0.3
Sample Extra Vol	25
Rinse Extra Vol	25
Mix Volume	0
Mix Time	0
Mix Flow Rate	0
Mix Height	0
Rack Def. pane (Figure 105)	
Settings for racks 1 to 5 must reflect the racks that exist on the system. If incorrectly set, severe damage to the needle (probe) can result.	
Main Control pane (Figure 106)	
Air Valve:	Off
Plunger:	Up
Syringe Volume:	Volume of the syringe on the Liquids Handler
Arm Z Scale (mm):	Set at installation
Calibrations pane (Figure 107)	No values at this time.

* These default values are designed for the most viscous solvents and will work for D_2O.

10. Calibrate **Probe Volume**, as described in "Calibrating Volumes and Flow Rates," page 345.
11. Acquire data on standard test samples, as described in "Acquiring Data on Standard Test Samples," page 354.

    This verifies that the VAST hardware and software are operational.
12. If desired, calibrate Sample Volume and the flow rate parameters as described in "Calibrating Volumes and Flow Rates," page 345.
13. Prepare your samples and transfer them to the sample wells in a sample rack. Be sure to note where each sample is located in the sample tray.

The VAST accessory is now ready for use with the VNMR automation software. Refer to the following procedures in this section:

- "To Set Up NMR Experiments for VAST," page 314
- "To Change Samples with VNMR," page 314
- "To Shut Down a VAST System," page 316

To Set Up NMR Experiments for VAST

If solvent suppression is not needed, only routine parameters such as `tpwr`, `pw90`, `d1`, and `sw` need to be optimized. The usual commands (`go`, `ga`, `au`) can be used to start acquisition.

If solvent suppression is needed, use WET and Scout, as described in section 10.2 "Solvent Suppression in VAST," page 317. Processing, displaying, and plotting of data obtained with VAST is described in section 10.3 "Processing, Displaying, and Plotting VAST Data Sets," page 323.

To Change Samples with VNMR

You can change samples manually or automatically.

Manually

Sample location is defined with a number (`loc=3`) or with a string (`vloc='G7'`), using the `loc` or `vloc` parameters.

To change samples and not run acquisition:

- In the current zone and rack—for example, location 3—enter:
  `loc=3 change`
  You can also set `vloc` to a value like `'A3'` or `'G7'` if you know the microtiter-plate address.
- In a different zone or rack—for example rack 1, zone 1, location 3—enter:
  `loc=3 vzone=1 vrack=1 change`

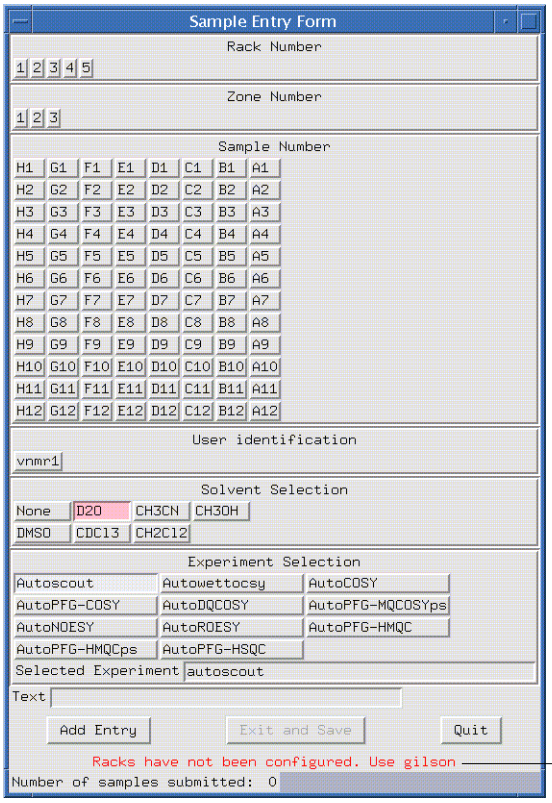
To change samples and run acquisition (`ga`, `go`, `au`, or `su`):

- In the current zone and rack—for example, location 3—enter:
  `loc=3 ga`
- In a different zone or rack—for example rack 1, zone 1, location 3—enter:
  `loc=3 vzone=1 vrack=1 ga`
  or
  `vloc='G7' ga`

Automatically with the enter Program

To change samples automatically with the `enter` program, use the following steps.

1. Type **`enter`** in the VNMR input window.
   The `enter` program prompts you for a file name that stores the information set by the `enter` program. You can also use the command `enter('file')`.
   The Sample Entry Form window appears, similar to Figure 77.
2. Select the desired samples in the window.


Sample Entry Form
Rack Number
1 2 3 4 5
Zone Number
1 2 3
Sample Number
H1 G1 F1 E1 D1 C1 B1 A1
H2 G2 F2 E2 D2 C2 B2 A2
H3 G3 F3 E3 D3 C3 B3 A3
H4 G4 F4 E4 D4 C4 B4 A4
H5 G5 F5 E5 D5 C5 B5 A5
H6 G6 F6 E6 D6 C6 B6 A6
H7 G7 F7 E7 D7 C7 B7 A7
H8 G8 F8 E8 D8 C8 B8 A8
H9 G9 F9 E9 D9 C9 B9 A9
H10 G10 F10 E10 D10 C10 B10 A10
H11 G11 F11 E11 D11 C11 B11 A11
H12 G12 F12 E12 D12 C12 B12 A12
User identification
vnmr1
Solvent Selection
None D2O CH3CN CH3OH
DMSO CDCl3 CH2Cl2
Experiment Selection
Autoscout Autowettocsy AutoCOSY
AutoPFG-COSY AutoDQCOSY AutoPFG-MQCOSYps
AutoNOESY AutoROESY AutoPFG-HMQC
AutoPFG-HMQCps AutoPFG-HSQC
Selected Experiment autoscout
Text
Add Entry
Exit and Save
Quit
Racks have not been configured. Use gilson
Number of samples submitted: 0

If you did not configure the racks you will see this message. Click on Quit and configure the racks.

Figure 77. VAST Sample Entry Form Window (enter program)

3. Click **Add Entry**.
4. Click **Exit and Save**.
5. Enter **`autogo`**

   You are prompted for the file set up by the `enter` program and for the name of a directory in which the automation data is to be stored.

   Enter the file name from step 1 and the name of a directory. You can also include the file and directory in the command by entering `autogo('file','directory')`.

   The automation run begins.

   The `autogo` command by itself uses a different set of parameters than the **Start Run** button on the LC-NMR pane, which is described below.

Automatically from the LCNMR/STARS Pane

A preferred alternative to using the `enter` program is to use the LCNMR/STARS pane (shown in Figure 78), which has the buttons **Sample Entry** and **Start Run**. This pane is automatically converted to a VAST Experiment Setup pane when you initialize VAST using the menu buttons or by typing VAST1D on the command line. Unlike other sample changer software, these buttons use the current parameter set as the starting conditions for the subsequent `Autoscout` acquisition, which is described in "To Set Up NMR Experiments for VAST," page 314.

The LC-NMR pane makes it easier to use automation.

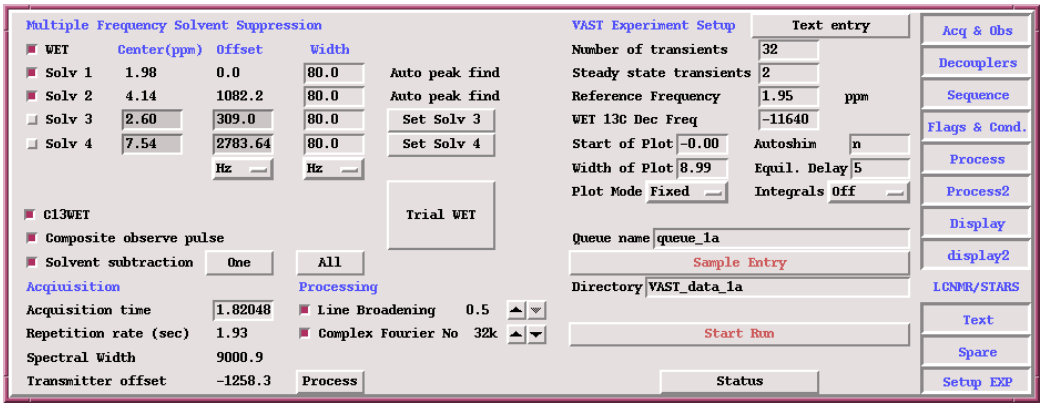


Figure 78. LC-NMR Pane for VAST

1. In VNMR click **Main Menu** > **Setup** > **Flow-NMR** > **Initialize VAST**.
2. Click the **LCNMR/STARS** tab.
3. Click the **Sample Entry** button.

   A Sample Entry window pops up.
4. Fill in the Sample Entry Form window.
   - **Peak Number** – select one or more peaks on which to run experiments.
   - **User Identification** – select the user.
   - **Solvent Selection** – select the solvent for the chosen peak(s).
   - **Experiment Selection** – select Autoscout, Autowettocsy, or both for the chosen peaks.
   - **Text** – enter information about the experiment, if desired.
   - When finished with this peak (or peaks), click **Add Entry**.

   When you are done adding entries, click **Exit and Save**.

   The sample specified by the Queue name file is run, and the data is stored in the specified directory.

After the automation run is started, you can click the Status button to bring up a status window.

Using the LC-NMR pane replaces the commands `enter`, `autogo`, `status`. Unlike the `autogo` command, however, the Start Run button saves the current parameter set (including solvent suppression parameters) and uses this for all subsequent acquisitions called for by the `autoscout` macro.

To Shut Down a VAST System

The following procedure to shut down the VAST system consists of: setting the `loc` parameter to the appropriate value and then flushing the sample out of the probe with the air valve.

1. Set the loc parameter to 0 or 'n' as follows:
   - To extract a sample from the NMR probe after a manual run (needle is in the injector port):

     `loc=0 change`

- To disable the Gilson Liquid Handler so samples are not changed inadvertently:
  loc='n'

2. In the VNMR input window, enter **gilson** to open the Liquid Handler window.
3. Click on the **Main Control** tab in the Liquid Handler window.
4. Clear the NMR probe of any leftover sample or solvent by setting **Air valve** to **ON**.

   Let this run for about two minutes or until you hear air hissing out of the injector port. Be prepared to wipe up any sample or solvent that seeps out of the injector port.

10.2 Solvent Suppression in VAST

Solvent suppression is invariably the first step needed for any VAST experiment. After the initial solvent suppression setup is done, however, you only need to click the Trial WET button in the VAST pane to perform further optimizations.

This section contains the following:

- "Setting Up Solvent Suppression," page 317
- "Troubleshooting Solvent Suppression," page 321
- "Evaluating Solvent Mixture Equilibration," page 322
- "Solvent Suppression: Background Information," page 322

Setting Up Solvent Suppression

1. Retrieve a parameter set appropriate for VAST by using either the menu system (**Setup** -> **Flow-NMR** -> **Initialize VAST**) or through the **rtp** command.
2. Regulate the probe temperature (preferably to a value close to ambient). The value of `temp` is displayed in the Acq & Obs pane.
3. Turn the lock off. Set **lockgain** and **lockpower** to zero.

   Turning off lock eliminates *lock pull* during each spectrum of a solvent gradient experiment and *lock jumps*. *Lock pull* is caused by the constantly changing HOD frequency during the signal averaging. *Lock jumps* occur if the concentration of the deuterated species drops below the detection threshold (see the Noise reject parameter).

   The sudden loss of lock, with its commensurate sudden change in field, is annoying during Trial WET setup operations. Even more critically, it can disrupt solvent suppression during a VAST run. Although turning the lock off is not mandatory, data is measurably better unless you are running a long experiment.
4. Tune the probe and adjust shimming if necessary.
5. Check if the global parameters `ref_pw90` and `ref_pwr` exist:
   ref_pw90?
   ref_pwr?

   or **click** on the **Sequence tab** and look at the **VAST sequence pane**, see Figure 79.
6. If `ref_pw90` and `ref_pwr` do not exist, create them:

```
create('ref_pw90','pulse')
create('ref_pwr','real')
```

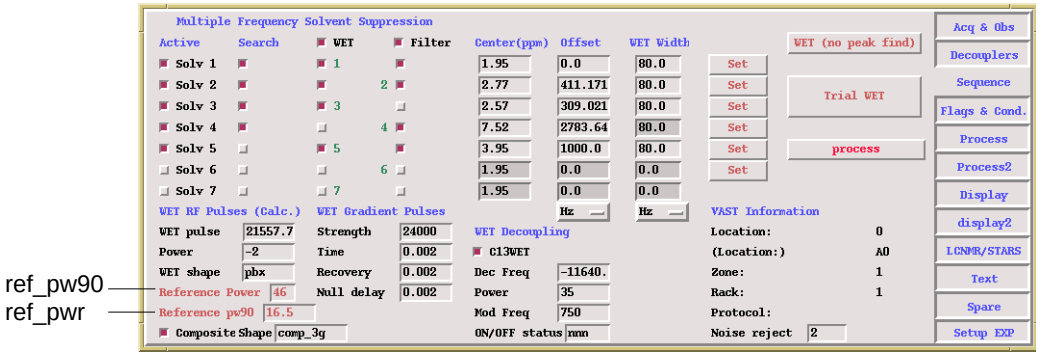


Figure 79. VAST Sequence Pane

7. If they do exist, make sure they are set correctly for the probe being used (e.g., `ref_pwr=44` and `ref_pw90=25`). The `ref_pwr` used must be within the linear portion of the amplifier's operational range. These values are used to calculate the power levels for the shaped pulses. Generally, pw90's determined at power levels that are 12 to 20 db below the level used for the observe pw90 are within the linear operating range of the amplifier.

8. **Click** on the **LCNMR/STARS** tab to open the **VAST pane** see Figure 80.

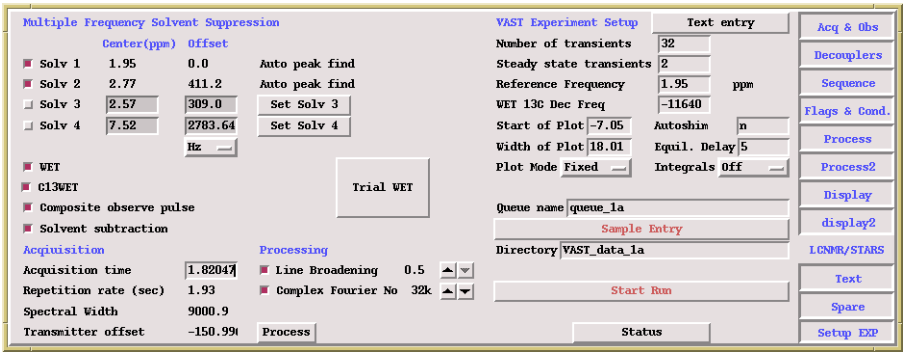


Figure 80. VAST Pane

9. **Click** on **WET** and **select: Solv 1**, **Solv 2**, **Solv 3**, or **Solv 4** to set the number of NMR signals (up to 4 signals using this pane see step 13 for suppression of up to 7 signals) you intend to suppress.

   The first NMR signal to be suppressed must be suppressed using Solv 1. If the spectrum contains two or more NMR signals that are to be suppressed, select Solv 2, Solv 3, or Solv 4 until the appropriate number of signals have been chosen. Solv 2 does not have to be selected to use Solv 3, or Solv 4. A more detailed discussion on how to determine the which signal suppression option to select when 2 or more signals are to be suppressed is given in step 13.

   The value of the Center and Offset for Solv 1 is determined automatically by searching over a wide band of frequencies.

   - If Solv 2 is not selected, the tallest peak in the spectrum is suppressed.
   - If Solv 2 is selected, the high field signal is assigned to Solv 1 (this may or may not be the tallest signal in the spectrum) and then the region of the spectrum that is to the low field side of Solv 1 is searched for the tallest signal in that region.

Center and Offset are determined automatically and displayed. These values are non changeable on this panel. These values can, however, be entered in the corresponding Sequence pane.

For Solv 3, or Solv 4 you must supply either a frequency for Center (in ppm) or a value for the offset from the carrier for Offset (in Hz). These values can be entered manually, or they can be extracted from a spectrum by placing a cursor at the appropriate location in the displayed spectrum and pushing the appropriate Set Solv button. When either Solv 3, or Solv 4 or both are selected, a narrow range of frequencies, about the set frequency is searched. The frequency range of the search is discussed in step 13.

Three additional NMR signals can be suppressed for a total of 7. The Sequence pane provides control of the suppression of these additional signals - see step 13.

10. **Select** the **C13WET** box to suppress the ^{13}C satellites of the solvent resonances.

    This assumes the values for Dec Freq, Power, and Mod Freq (located in the Sequence pane) have been properly set. The ON/OFF status is typically nnn, although nny may be useful if the size of the residual 13C satellites are a problem.

11. Click on the **Acq & Obs** tab to open the Acq & Obs pane see Figure 81.

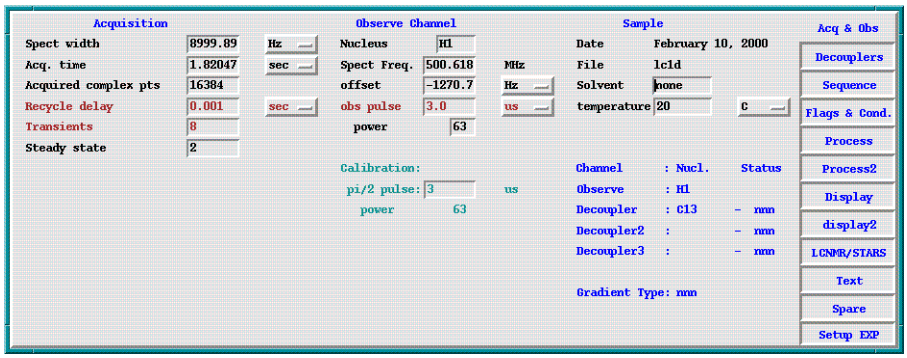


Figure 81. VAST Acq & Obs Pane

12. The **obs pulse** and **power** should be set to value that produce `pw90` that is as short as practical. In general, the shorter the better, although 3.0 or 4.4 microsecond `pw90`s are particularly useful. see step 14.

13. VAST Multiple Frequency Suppression Options.

    a. **Click** on the **Sequence tab** to open the **Sequence pane** for suppression of more than four signals.Select up to 7 signals using the LCNMR/STARS pane. One (must be Solv 1) or two (must be Solv 1and Solv 2) frequencies can be searched for automatically. Five additional frequencies can be searched for over a narrow range based on the value of the center frequency. In the Sequence pane, up to 7 signals can selected. Solv 1 must be active and search selected if two or more signals will be suppressed.

    Both Solv 1 and Solv 2 (see step 9 for description of Solv 1 and Solv 2 frequency search) use algorithms for finding the signal frequency that are different from the algorithm used by Solv 3 through Solv 7 to locate the signal frequency. If the search option is active for any one of four signals, Solv 3 through Solv 7, the search is restricted to an approximate range of + or - 0.2 ppm about the value displayed in the Sequence pane for the Center

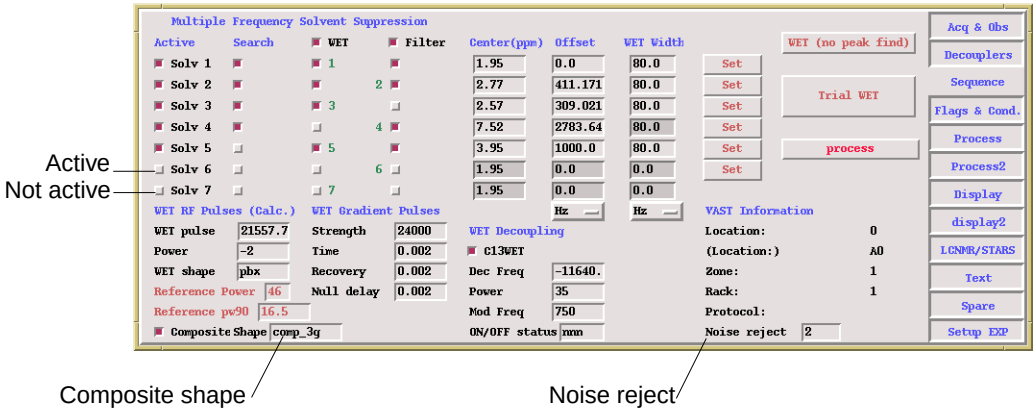


Figure 82. VAST Sequence Pane and Frequency Suppression Options

frequency. The Center frequency can be either entered manually or set by placing the cursor over the signal and pressing any one of the SET buttons. The value of Offset is calculated automatically.

b. Specify **acquisition and processing options** for each signal. Each **Solv** selection, see Figure 82, has four choices:

- **Search** for a signal.
- **Suppress** a signal using **WET.**
- Apply post acquisition signal suppression **filtration.**
- Specify the **Wet Width** of the signal suppression.

Each choice may be applied individually or in any combination. The choices are only active if the Active button for a given Solv is RED, see Figure 82. Currently the software limits post acquisition filtration to a maximum of 4 signals (more then four then filter buttons to may be set to active in the tcl pane). If five or more filter buttons are active, only the first four active Solv with filtration selected will be active.

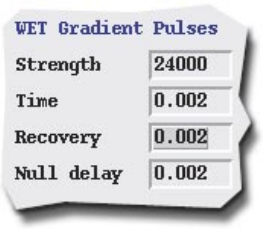
Wet Width is typically set to 80 Hz (for each solvent frequency; this corresponds to about a 20 msec pulse). You can adjust these entries to larger or smaller values to generate a larger or smaller bandwidths of suppression for each signal (as desired). This value of Width determines the duration of the WET pulse, which influences the power of the WET pulse (both parameters being displayed in the Sequence pane).

Pressing the **Wet (no peak find)** button will execute scan using the parameters as they appear in the Sequence window. WET suppression and or post acquisition Filtering will only be applied to those signals that are Active and have either or both of these options selected.

Pressing the **Trial WET** button will execute a search for each active signal with search enabled and apply all other options that are enabled for each active solvent.

14. **Select Composite observe pulse** in the **LCNMR/STARS** pane (Figure 80) if you can achieve a `pw90` of 3.0 or 4.4 µs. This will produce better looking data. Comp shape in the Sequence pane (Figure 82) must also be properly set (for pw=3.0 use comp_3pos and for pw=4.4 use comp_44g).

15. In the **Sequence** pane, the parameters under WET Gradient Pulses are typically set as follows:



Label	*Value*	*Parameter Equivalent*
Strength	**24000**	`gzlvw`
Time	**0.002**	`gtw`
Recovery	**0.002**	`gswet`
Null delay	**0.001**	`dz`

Because the solvent resonances are often left upside down in relation to the peaks of interest, the Null delay can be optimized to a value between 0 and 0.005 to catch the signals as they pass through zero (in analogy to the `WEFT` experiment).

16. Set **Noise reject** to **3** in the **Sequence** pane, see Figure 82.

The Noise reject parameter affects the determination of the frequencies for solvents 2 through 7, but not solvent 1. Set Noise reject to a higher or lower number as appropriate:

- To maximize the reliability of peak-finding for large solvent resonances, set Noise reject to a larger number (5 to 10).
- To detect smaller resonances, set Noise reject to a smaller number (1 to 3).

Noise reject does not accept values of less than 1.

- If Noise reject is set too small, the Scout Scan™ incorrectly detects an incorrect frequency to suppress (e.g., a noise spike or a tall sample resonance).
- If Noise reject is set too large, the Scout Scan™ does not detect the peak you intend to suppress (in which case Scout Scan™ sets the frequency to the last value used, which might not be accurate for the current sample), although this is a useful way to suppress a fixed frequency or a very tiny signal.

17. Click on the **Trial WET** button in the **VAST** pane, Figure 80, to perform further optimizations. This starts the Scout Scan process. Two spectra are produced:

- The first spectrum is a one-transient no-solvent-suppression 1D spectrum (full spectral width). The NMR software spends a few seconds analyzing this data, creating the appropriate shapes, and setting up parameters.
- The second spectrum, a signal-averaged data set, is acquired using the parameter optimization that was done after the initial acquisition completed. The data is automatically Fourier transformed and displayed as the second spectrum.

Troubleshooting Solvent Suppression

If the suppression does not look as good as it should, the cause might be one of the following:

- PFG gradients are not properly connected, turned on, or configured (check the values of `gradtype` and `pfgon`).
- Probe is not well rinsed out.
- Shims are not optimized. Note that the shims can change significantly between acetonitrile and methanol solvent mixtures.
- Parameters `ref_pwr` and `ref_pw90` are incorrect for the probe currently in use.

Evaluating Solvent Mixture Equilibration

The NMR chemical shifts of the solvent signals change as a function of mobile phase composition. You should always allow the pumped LC solvent mixture to equilibrate before starting an NMR run. One way to evaluate this is by doing the following:

1. In the **VAST** pane, press the **Trial WET** button.
2. After Trial WET finishes, check the value of transmitter offset as well as the **Offset** values for any selected **Solv 2**, **Solv 3**, and **Solv 4** entries.
3. Repeat 1 and 2 until these values remain constant. The system is equilibrated when these numbers do not change after you run Trial Wet.

   The offsets for Solv 1 and Solv 2 are usually determined by the Scout Scan. They are not directly changeable by the user in the VAST pane, but manual entry is possible in the Sequence pane.

Solvent Suppression: Background Information

For VAST, the VAST pane and the Sequence pane are of the most interest:

- Virtually all routine VAST operations are available from the VAST pane.

WET Experiments

Using a modified form of the WET (Water Eliminated through Transverse gradients) experiments, the peaks of (dis)interest are excited with a selective 90˚ pulse, using a WET waveform that has been convoluted with one or more frequencies (SLP). This pulse is then followed with a gradient to dephase the undesired signals, and the process is repeated multiple times. The WET shape has been found to be a suitable choice for VAST applications.

WET Shapes

The WET shape used (set to `pbx` in the Sequence pane) is calculated on-the-fly by the Pbox software package, which is now resident within VNMR. This shape is recalculated many times during an VAST experiment. For this reason, the single shape name `pbx` is used by the pulse sequence although the shape is recalculated for each use.

While a number of basic shapes can be used to apply selective pulses to solvent lines, there are always trade-offs. Longer pulses are more selective, but require more time (which is of the essence, particularly in flowing systems). WET (based on SEDUCE-1) is a shape taken from the literature that has been found to be very effective in pulsed field gradient solvent suppression experiments.

One of the two shaped pulses `comp_3g.RF` or `comp_44g.RF` is used when the Composite observe pulse box in the VAST pane is selected. The user can define which of these is used in the Comp shape field in the Sequence pane.

Related Parameters

Solvent suppression is achieved not only by the pulse sequence, but also (in part) by software processing. Software processing is most frequently accomplished by using `wftlc` after the parameters `ssfilter` and `ssntaps` (and perhaps `ssorder`) have been set to reasonable values (typically, `ssfilter and solvent suppression:LC-NMR ssfilter` is set to about `80`, `ssntaps` is set to about `251`, and `ssorder` is not used).

Another parameter of interest is `sslsfrq`. This parameter shifts the location of the filter notch (in Hz from `tof`). Often, the quality of solvent suppression can be improved somewhat by setting `sslsfrq` to a small positive number (e.g., `sslsfrq=3`). This parameter can also be arrayed to produce multiple filter notches within the same spectrum. Selection of which signal to which the notch filter is applied is made in the **Sequence** pane. Any four of the available seven signals may be selected. NOTE: the filter will be applied only to the first four signals if more then four are selected.

10.3 Processing, Displaying, and Plotting VAST Data Sets

Spectra obtained during a VAST automation run can be processed and displayed in different ways:

- Individually, using standard VNMR 1D processing.
- As a pseudo 2D data set.
- As an array of 1D spectra.
- Using Combiplate

Processing the spectra as a pseudo 2D data set or as an array of 1D spectra facilitates the inspection of all the spectra at the same time.

Combiplate provides a graphical representation of the samples based predefined conditions.

Creating a Pseudo 2D Data Set

The individual spectra from a VAST automation run can be merged into a pseudo 2D data set. Two related display macros are used to create the pseudo 2D data set:

- `vastglue`
- `vastglue2`

The criteria for selecting which macro to use is determined by the value of the parameter `autoname`.

- If `autoname=''` (*the default and preferred value*) at the beginning of an automation run, construct the pseudo 2D data set with `vastglue`.
- If `autoname='<user defined>'` at the beginning of the automation run, construct the pseudo 2D data set with `vastglue2`. The special case, `autoname='<user defined>'`, is not as user friendly as the `autoname=''`. The macro `vastglue2` must be hard coded to accommodate the user defined name.

vastglue

The macro `vastglue` accepts either of two pairs of arguments or no argument. The two pairs of arguments for `vastglue` are: `rack,zone` and `glue order,plate`.

You must provide two arguments or no argument. The default, rack=1 zone=1, is specified as `vastglue(1,1)`. Where the argument `rack,zone`, or the default is used, the glue order is determined from the `doneQ` file. This is the *default and preferred* gluing option. Part of a `doneQ` file is shown Figure 83.

The order in which each spectrum was acquired is specified by the entry on the line `DATA`. In this example, the micro titer plate was defined with each micro titer well having a number (1 through n). The first two digits are the well number and the second two digits specify the experiment. In an automation run, the first spectrum is the *autoscout* spectrum and the second spectrum is the optimized *wet solvent suppressed* spectrum. The macro "glues" together the even numbered spectra to create the pseudo 2D data set that can be saved using `svf('filename')`.

The `doneQ` file, Figure 83, is written by the automation macro into the same directory as the spectra. Before starting a VAST automation run two directories were specified, one in the Queue name field and one in the Scout directory field, Figure 84. The Scout directory field specifies where the spectra `doneQ`, and other files are to be stored. If you are moving data sets you should move this entire directory, not just the spectra. In the other directory are a Queue file and directory used by automation software during the automation run.

```
SAMPLE#: 1
    RACK: 1
    ZONE: 1
    USER: vnmr1
   MACRO: autowet
 SOLVENT: D2O
    TEXT: A Micro titer plate mix ACDMT
 USERDIR: /export/home/vnmr1/vnmrsys
    DATA: 0101
  STATUS: Complete
------------------------------------------------------------------------
-
  SAMPLE#: 1
    RACK: 1
    ZONE: 1
    USER: vnmr1
   MACRO: autowet
 SOLVENT: D2O
    TEXT: A Micro titer plate mix ACDMT
 USERDIR: /export/home/vnmr1/vnmrsys
    DATA: 0102
  STATUS: Complete
------------------------------------------------------------------------
-
  SAMPLE#: 2
    RACK: 1
    ZONE: 1
    USER: vnmr1
   MACRO: autowet
 SOLVENT: D2O
    TEXT: A Micro titer plate mix ACDMT
 USERDIR: /export/home/vnmr1/vnmrsys
    DATA: 0201
  STATUS: Complete
------------------------------------------------------------------------

  SAMPLE#: 2
    RACK: 1
    ZONE: 1
    USER: vnmr1
   MACRO: autowet
 SOLVENT: D2O
    TEXT: A Micro titer plate mix ACDMT
 USERDIR: /export/home/vnmr1/vnmrsys
    DATA: 0202
  STATUS: Complete
------------------------------------------------------------------------
-
  SAMPLE#: 3
```

Figure 83. Partial `doneQ` File

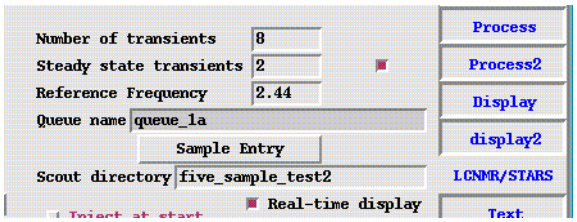


Figure 84. Queue Name and Scout Directory Fields

The `glue order,plate` option allows the glue order to be defined by the user when the data set is glued rather than using the `doneQ` order. This method will produce a pseudo

2D data that can be displayed with as a contour map, color map, or stacked plot. The association of the well number with the spectrum is lost and renders the display macro, `dsvast`, less useful (although still functional). The `plat_glue` program (see "Defining a Custom Display Order with plate_glue" on page 327) allows the user to redefine the order in which the spectra in a pseudo 2D data set are displayed. Since this is a post-gluing operation, the association between the well and the spectrum can always be determined.

vastglue2

The `vastglue2` macro artificially reconstructs a 2D data set from a series of 1D data sets having similar filenames. It is crucial to ensure that the format of the file names of each of the 1D data sets is identical. The `vastglue2` macro reads each 1D file in succession, and adds it to the previous data, but in a 2D format. It assumes that the wells will be named using a predefined format, for example:

```
autoname='filename_R%RACK:%_Z%ZONE:%_S%SAMPLE#:%_'
```

This definition must be hard coded in the macro by the user. A different version of the macro must be written for each variation of the autoname format.

Creating a pseudo 2D DATA Set Using vastglue

This procedure creates a pseudo 2D data set for rack 1 zone 1. See "vastglue" on page 324 for other rack and zone combinations.

1. Start `VNMR`.
2. Join any experiment other than exp5
3. Change directories to the directory containing the VAST experiment FIDs.
4. Type `vastglue`.

   If the data was acquired other then in rack1 zone 1, recall the first spectrum and then type `vastglue(rack,zone)`.

   The gluing process takes place in exp5. Once the process is finished the glued data set is returned to the original experiment.
5. The pseudo 2D data set can be saved using the standard command, `svf('filename')`.
6. If the data set was acquired with fn>65k, set fn to a value ≤ 65 k before processing the data set.

There are several glue order templates provided with VNMR, the default is to use the done Q if no glue order is specified. Glue templates are found in `~/vnmr/user_templates/glue`.

Processing, Displaying, and Plotting Glued VAST Data

Process the glued data similarly to a spectrum from a 2D experiment.

1. Set the display and display orientation, enter:
   ```
   f full trace='f2'
   ```
2. Set the `'f1'` axis label:
   ```
   setwell
   setvalue('sw1',1,'processed')
   wf1=100 dconi
   ```

This example is for a 96 well data set. The setwell macro changes the label of the `f1` axis to “well number” and `setvalue('sw1',1,'processed')` labels each trace with the well number corresponding to that sample's spectrum. The number of traces displayed is set using `wf1=100 dcon`.

3. Enter **`pmode='full'`**

   If `pmode='full'` is set before `wft1da`, the 2D spectrum can be phased as needed and redisplayed using `dconi` without transforming all the spectra.

4. Enter **`wft1da`** to process the glued data.

5. Display the data set as a color map, a contour plot, or as a stacked plot.

   The standard 2D display options, vs2d, trace, projection, etc. all apply. For more flexible data presentation, use the processing macros `dsvast`, `dsvast2d`, and `vastget` and the plotting macros `plvast`, `plvast2d` and `plvastget`.

   - To generate a `dss` stacked display, enter `vastget`.
   - To display on spectrum, enter `vastget('well#')`.
   - To display selected spectra, enter:

     va`stget('well#','well#')`

     where `'well#','well#'` is an arbitrary list of one or more sample wells. The names of the wells are defined as A1 through H12 and are the names attached to each spectra by `dsvast` or `plvast`. The spectra are displayed or plotted as a `dss` stacked display.
   - To display spectra `1` through `arraydim`, use the `dsvast` macro.

6. Plot the data in the same way you would plot a true 2D spectrum.

   For more flexible data presentation, use the plotting macros `plvast`, `plvast2d` and `plvastget`.

   - Plot an individual spectrum with `pl`.
   - Plot a series of spectra with fixed intervals or all the spectra using:
     `plww(<start,finish,step> or <'all'>)`
   - To plot spectra `1` through `arraydim`, use the `plvast` macro.

VAST display macros, `dsvast` and `vastget`, and their corresponding plotting macros, `plvast` and `plvastget`, present or extract information from the `wft1da` or `wft` processed pseudo 2D data. The macro `dsvast` (or `plvast`) default to display (or plot) spectra 1 through `arraydim` (the number of spectra in the pseudo 2D data set) and labels each spectrum with the name of the sample well. A preset naming convention is used: rows A through H and columns 1 through 8. The spectra are labeled A1 through H8. These macros accept one or two arguments to determine the format of the display (`dsvast`) or plot (`plvast`):

```
dsvast<(display order),(number of columns displayed)>
plvast<(display order),(number of columns displayed)>
```

- If no arguments are given the default will display will 8 columns of spectra.
- If the `arraydim of glueorderarray` is ≤ 8, one row of spectra is displayed.
- If `dsvast` or `plvast` is provided with one argument, the argument is defined as the number of columns to be displayed. If two arguments are provided, the first argument, display order, must be the name of the file containing the glue order and the second argument is the number of columns displayed.

Defining a Custom Display Order with `plate_glue`

To define a display order that is different from the order defined in the doneQ file, use the `plate_glue` program.

1. Start `plate_glue` from a terminal window by entering:

   `plate_glue &`

   The plate glue window appears, similar to Figure 85.

2. Select the wells you want to display.

   A well turns gray when selected. As you select more wells, a red line joins the grayed out wells.Wells do not have to be adjacent to each other.

   - To select a well, click on it.
   - To deselect a well, right-click on a selected (grayed) well.
   - To select a row, partial row, column, partial column, or diagonal grouping of wells, click and hold the left mouse button on the first well, drag to the last well in the selection, and release the mouse button.

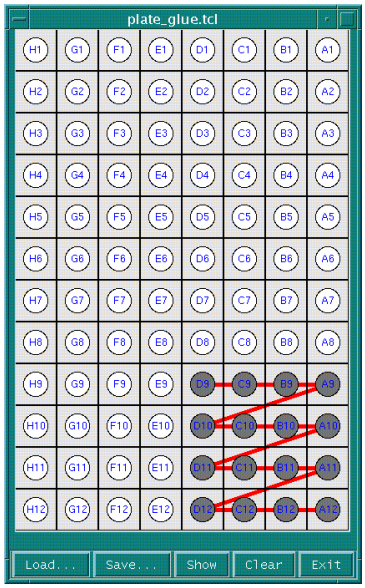

plate_glue.tcl
H1 G1 F1 E1 D1 C1 B1 A1
H2 G2 F2 E2 D2 C2 B2 A2
H3 G3 F3 E3 D3 C3 B3 A3
H4 G4 F4 E4 D4 C4 B4 A4
H5 G5 F5 E5 D5 C5 B5 A5
H6 G6 F6 E6 D6 C6 B6 A6
H7 G7 F7 E7 D7 C7 B7 A7
H8 G8 F8 E8 D8 C8 B8 A8
H9 G9 F9 E9 D9 C9 B9 A9
H10 G10 F10 E10 D10 C10 B10 A10
H11 G11 F11 E11 D11 C11 B11 A11
H12 G12 F12 E12 D12 C12 B12 A12
Load...
Save...
Show
Clear
Exit

Figure 85. `plate_glue` Window and `e4x4` glue order.

3. After the display order is defined, click the save button and save your custom glue order file in the `~/vnmrsys/templates/glue` directory with a file name of your choice. In this example the glue order file name is e4x4.

   The display and plotting macros, `dsvast` and `plvast`, look in `~/vnmrsys/templates/glue` for the glue order file.

Examples of Plots of a VAST Data Set

The plots in this section represent the data from a 96-well titer plate in zone 1, rack 1.

Figure 86 shows an array of 96 1D spectra plotted using `plvast` with no arguments.

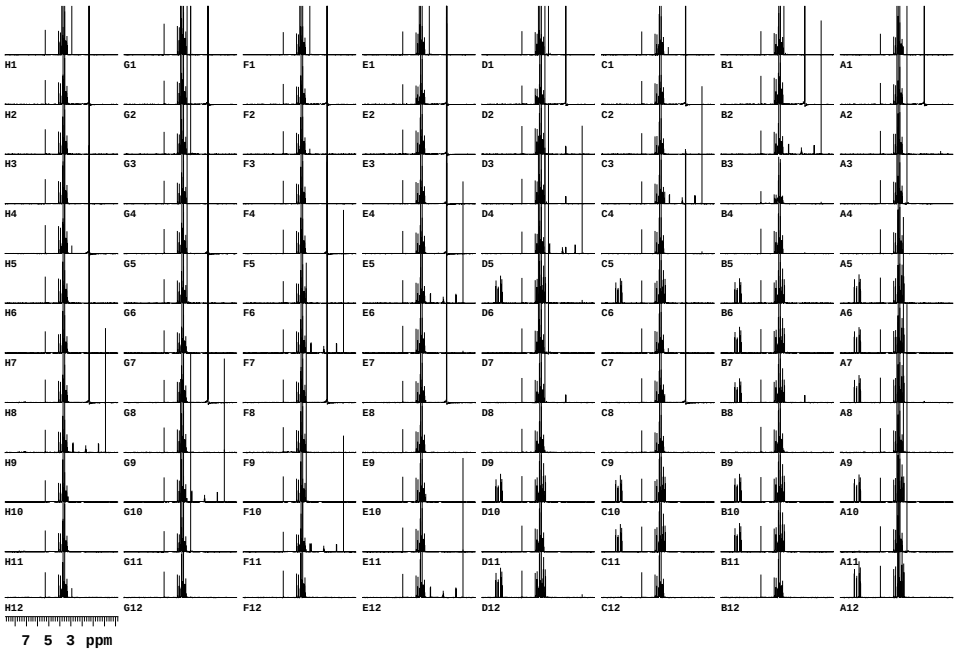


Figure 86. Array of 1D Spectra from a VAST Data Set

Figure 87 shows a plot of 16 spectra from the lower right corner. The `plate_glue` program was used to create a `glue` file called `e4x4` and the spectra were plotted using `plvast('e4x4',4)`. This produced a 4x4 matrix using the `glue` file `e4x4` and specifying the number of columns as 4.

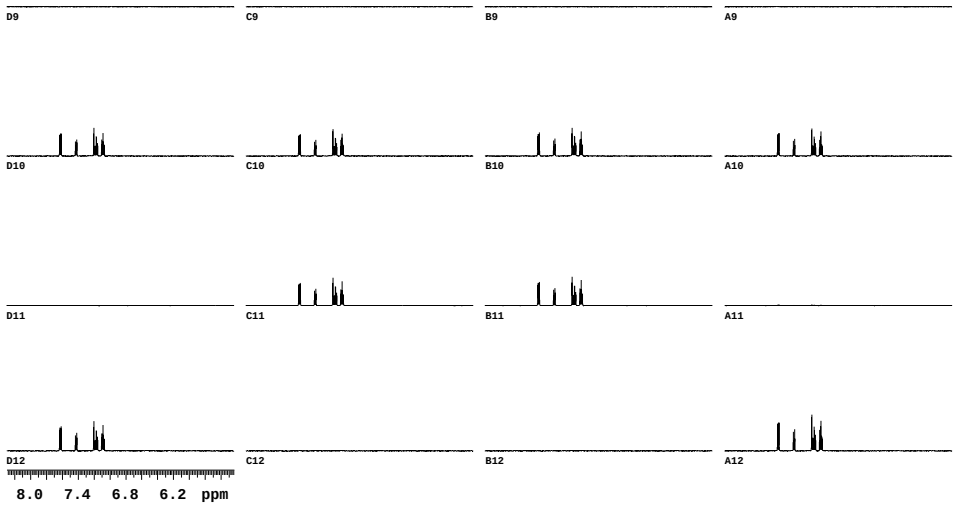


Figure 87. Plot of a Subset from a 96-Well Sample Plate

Figure 88 shows a pseudo 2D contour plot of the VAST data set.

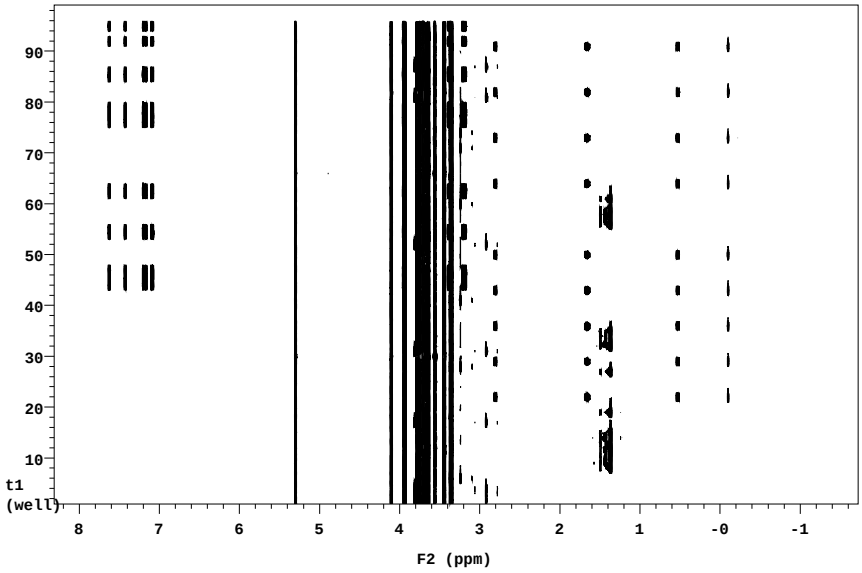


Figure 88. Contour Plot of a VAST Data Set

The pseudo 2D in Figure 88 was created using `vastglue` with no arguments. The `f1` axis of the plot is modified to display the well numbers by typing the following on the VNMR command line:

```
setwell
setvalue('sw1',1,'processed')
wf1=100 dconi
```

Pseudo 2D data sets processed with `wft1da` can be displayed and plotted using the same tools that are used for a typical 2D data set. The plot in Figure 88 was created using `pcon('pos',8)`.

Figure 89 shows the same data plotted as a stacked spectra using the `pl2d` macro.

Figure 90 shows the spectrum of the sample in well F6, extracted with `vastget('F6')` and plotted with `plvastget('F6') pap pscale page`.

Summary of VAST Display and Plot Options

To display or plot VAST microtiter plate data, you have the following options:

- Stack of 96 spectra in the output bin of the plotter. This is the normal output from an automation run.
- 8 x 12 matrix display of the full spectrum of each compound (similar to Figure 86). This display gives you the feeling of the entire plate at once, including perhaps approximate reaction yields, but little specific structural information. Use the `vastglue`, `dsvast`, and `plvast` macros.
- 8 x 12 matrix display of one specific spectral expansion of each compound, showing an interesting region (e.g., 6.5 to 9 ppm or 0.5 to 3 ppm). This display gives you more specific information about the chemical functionality and diversity available in the plate. Use the `vastglue`, `dsvast`, and `plvast` macros.

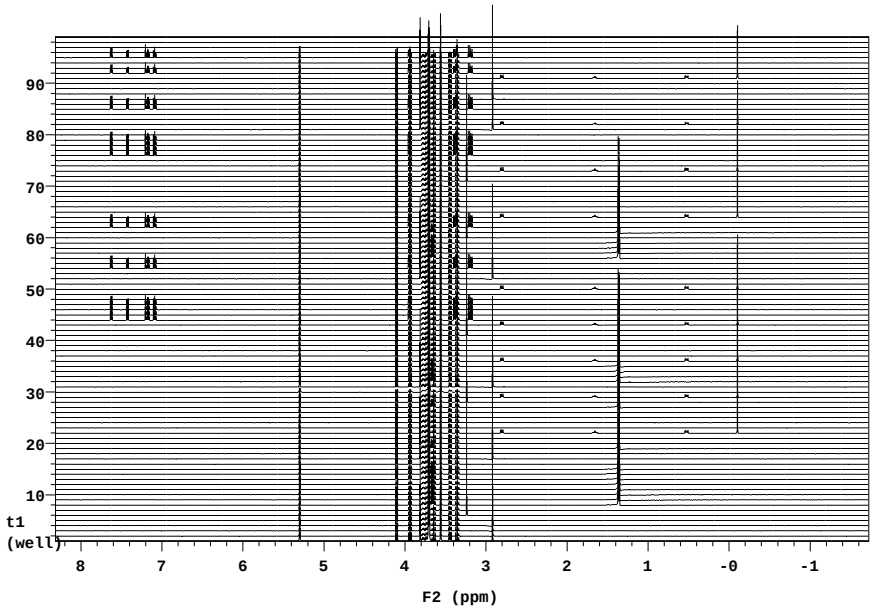


Figure 89. Stacked Plot of a VAST Data Set

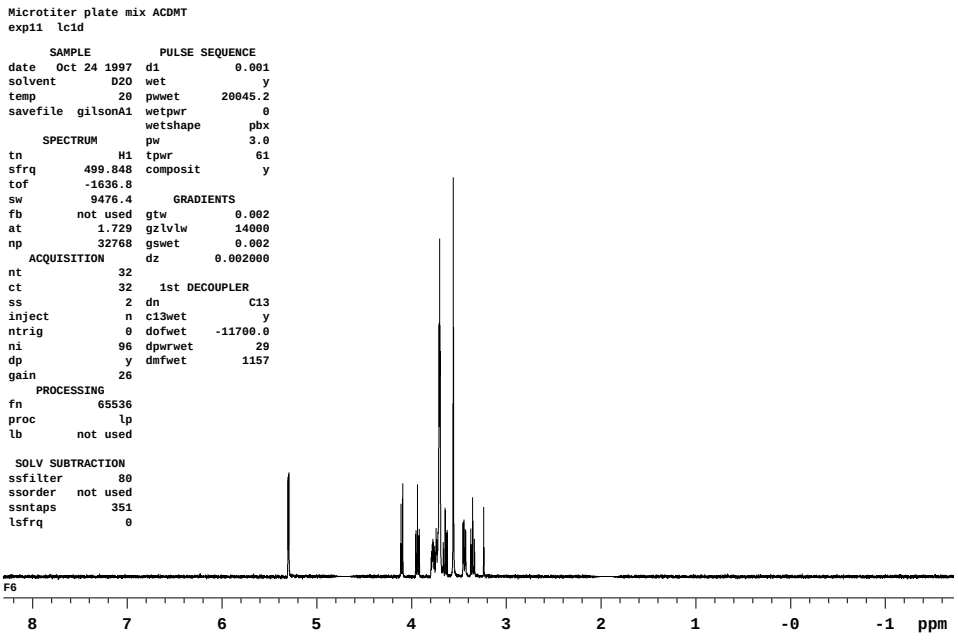


Figure 90. Spectrum of the Sample in Well F6

- 8 x 12 matrix display of one specific spectral expansion, where the expansion only includes a reference (control) resonance (e.g., TMS). This display allows you to calibrate concentration or to verify the quality of each injection. Use the `vastglue`, `dsvast`, and `plvast` macros.
- 8 x 12 matrix display of the integral of one specific spectral expansion of each compound, showing an interesting region (e.g., reference resonance, 6.5 to 9 ppm, or 0.5 to 3 ppm). This display can give a better picture of quantitation, especially if the

peak amplitudes are affected by differing NMR resolution for each well. Use the `vastglue`, `dsvast`, and `plvast` macros.

- To show a contour plot of the entire plate (similar to Figure 88), enter `trace='f2' wft1da`. You can look at each spectrum using the *trace* display to scan through each *increment* as desired. You can expand around either a given chemical shift range, or a certain number of wells (a row or column, depending upon the orientation of the plate). Use the `vastglue`, `dsvast`, and `plvast` macros.
- To view region intensities by colors and color densities, use the CombiPlate window, which is described in the next section.

10.4 Using CombiPlate to Analyze Data

CombiPlate allows you to view integral region intensities by colors and color densities. The CombiPlate window provides a map of the microtiter plate, which allows you to click on individual sample wells to bring up a spectrum in the VNMR display window.

Preparing VNMR Data For Analysis Using CombiPlate

The information displayed by *CombiPlate* is obtained from a pseudo 2D data set that was previously created and saved.

1. Create the pseudo 2D data set.

   See "Creating a Pseudo 2D Data Set" on page 323 for instructions on creating a pseudo 2D data set. The data set should be saved with all the spectra properly phased and referenced.

2. Change to the directory containing the VAST pseudo 2D data set to be used to construct the *CombiPlate* display.

3. The load the pseudo 2D spectra into the current experiment and process the spectra (*wft dsvast* is the most convenient processing and display method). Use any experiment except experiment 5. Experiment 5 is used by various macros during the processing steps. The pseudo 2D set will supply the data to be displayed by CombiPlate.

4. Start CombiPlate from the VNMR command line by typing:
   `combi_preproc.`

5. The macro will process the data in exp5 and display exp5 when it starts the processing. When the processing is completed, a spectrum representing the sum of all spectra is displayed in exp5. Use this spectrum is used to set the integral regions. Adjust the phase if necessary. Only the regions for the integration need to be set at this time so perfect phasing is not necessary. Adjust the amplitude of the integral as needed. Tilt and Level do not need to be critically adjusted.

6. Define up to three integral regions using the sum of spectra spectrum created in step 4.

7. Join the original experiment and move the parameters from exp5 to the original experiment.

8. Type `mp(5,'original exp')`

9. Type *dsvast* to display all the spectra then, using *vastget('well')* (each well is defined [A->H][1->8] e.g. F8), select one having the widest range of resonances.

10. Carefully **phase** this spectrum, **check** the **integral Lvl/Tlt**. If necessary correct the integral Lvl/Tlt to give proper integration. DO NOT change the integral regions.

11. Type `nlivast(`*`number of wells analyzed`*`)`. The *number of wells analyzed* must be an even number. The file ASCII text file, *integ.out*, will be created in the current experiment's directory (`~/vnmrsys/exp(#)/integ.out`). CombiPlate uses the data in *integ.out* to create the display. If you intend to continue the analysis at a later time and you will be running CombiPlate from a terminal window, copy this file to the directory containing the VAST data. Renaming the file, although not required, is suggested. Each time *nlivast* is run it creates another *integ.out* file. Any previous copies of this file in the current active directory are overwritten.

Data Analysis Using CombiPlate And VNMR

Complete the section, "Preparing VNMR Data For Analysis Using CombiPlate," page 331 before continuing with the instructions here.

1. On the VNMR command line type:

   `combishow(1,2,3).`

   This assigns region (1) as red, region (2) as green, and region (3) as blue and starts the CombiPlate display using the data in (`~/vnmrsys/exp(#)/integ.out`). The initial colors displayed are from a synthetic data included with CombiPlate and may not be correct, see Figure 91 and "Checking And Fixing The Color Map.," page 336. Correcting the colors is *not* necessary at this time.

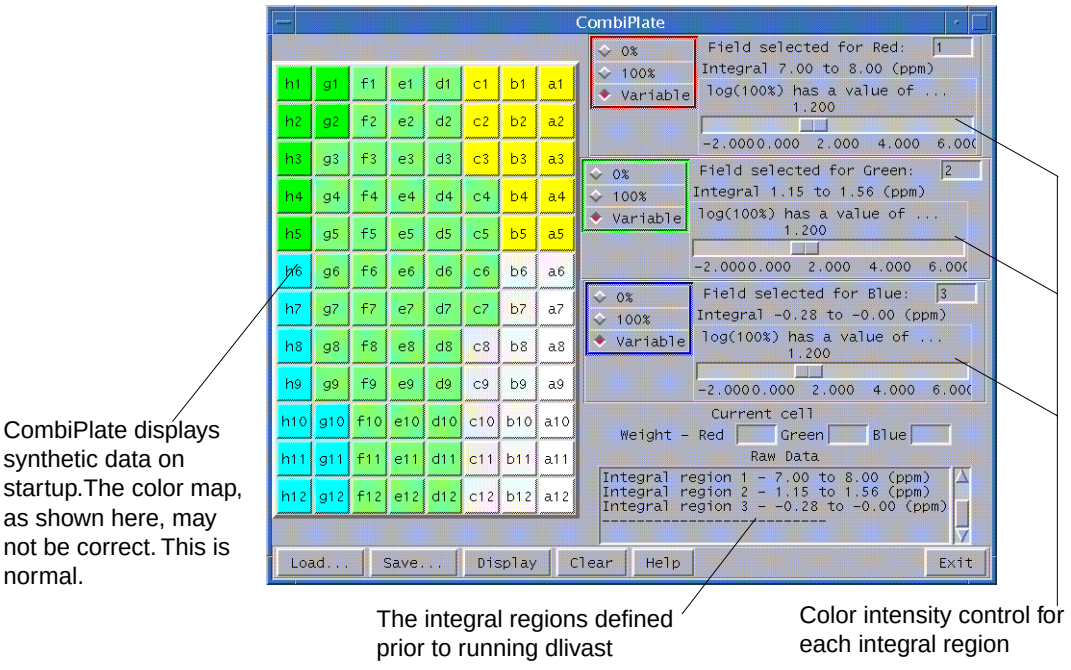

CombiPlate
Field selected for Red:
Integral 7.00 to 8.00 (ppm)
Field selected for Green:
Integral 1.15 to 1.56 (ppm)
Field selected for Blue:
Integral -0.28 to -0.00 (ppm)
0%
100%
Variable
log(100%) has a value of ...
1.200
Current cell
Weight - Red Green Blue
Raw Data
Integral region 1 - 7.00 to 8.00 (ppm)
Integral region 2 - 1.15 to 1.56 (ppm)
Integral region 3 - -0.28 to -0.00 (ppm)
Load...
Save...
Display
Clear
Help
Exit
CombiPlate displays synthetic data on startup.The color map, as shown here, may not be correct. This is normal.
The integral regions defined prior to running dlivast
Color intensity control for each integral region

Figure 91. CombiPlate Started by **combishow** From **vnmr**

2. **Click** the **OK** button on the **Welcome!** window to close when you finish reading its contents.

3. **Click** the **Display** button to load the data into the CombiPlate window. Depending upon the values of the integrals you may or may not have any colors displayed.

- If no colors are displayed set all the *log(100% color intensity)* slider bars to (log(100%)= - 2.000) and **Click** the **Display** button.

4. Set the color intensity for each region.

   The integral regions defined in step 6 of "Preparing VNMR Data For Analysis Using CombiPlate," page 331 are displayed in the scrolling text box at the at the bottom of the CombiPlate window and in each color field. Color intensity for each region is controlled independently. Each region has two preset values, 0% and 100% and a variable. If the variable option is selected the intensity of the color can be related to the integral area.

   - If the *log(100% color intensity)* equals the largest integral value the color intensity is related to the integral area.
   - If *log(100% color intensity)* is set to a value equal to or less then the smallest positive non zero integral value for that region the intensity is not related to integral area. The color is binary, on or off. In this case the color is turned on (a non zero positive integral) or it is off (integral is either zero of negative).

   a. Set all the *log(100% color intensity)* slider bars to (log(100%)= - 2.000). This will set up a binary condition. The display in Figure 92 is set up to turn on the color of a given region only if there is a positive integral for that region.

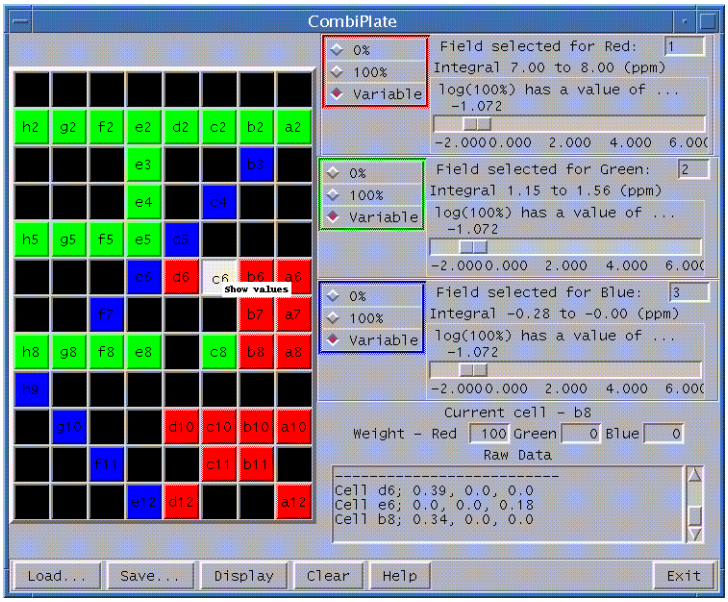


Figure 92. CombiPlate Display - Binary Display For Each Region

   b. **Click** on the **Variable** radio button.

   c. **Click** the **Display** button.

   d. To use the intensity of the color as a measure of the integral area of a region, move the slider bar for that regions to the right. **Click** on the **Display** button. If all the color for that region diappears, you moved the bar to far - move it back to the left. Select a value that provides a display with both the smallest integral value and largest integral value represented. Repeat for each region as desired. The display does not automatically update as the slider bar is moved. You must **Click** on the **Display** button to view all changes.

5. Individual spectra can be examined by selecting and clicking on the cell of interest. In Figure 92 cell c6 has been selected. The cell is white and the label “Show values” is displayed. The spectrum and inset spectral regions are displayed in the vnmr window. This feature is active only when vnmr is active and CombiPlate is started from combi_show on the vnmr command line.

6. Save the CombiPlate display by clicking the **Save** button.

   In the CombiPlate save file window, Figure 93, you specify the directory and file name and which region or regions the file is to contain. A data set that was analyzed with all three regions active can be saved in three independent files, on for each region by selecting one region. You must select one or more colors. If you do not select any colors a message “No colors selected” is displayed and no further actions takes place.

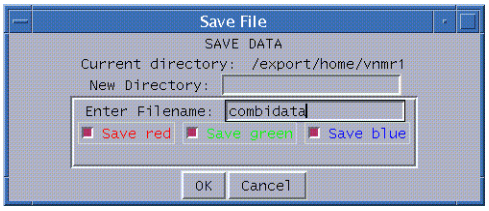


Figure 93. CombiPlate Save File Window

Analyzing Data Using CombiPlate Without VNMR.

When CombiPlate is started from a terminal window, clicking on a cell will display the integral information for that well in the scroll box at the lower right corner of the CombiPlate window. No spectra will be displayed, even if VNMR is running.

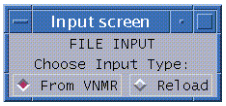
- Complete the instructions in “Preparing VNMR Data For Analysis Using CombiPlate,” page 331 before continuing.

CombiPlate loads a synthetic data set that will give it an evenly graded color scheme running from green in the top left to yellow (top right), light blue (bottom left) and white (bottom right). If you do not see a smooth, even gradation of color, there are other programs running that are using colors requested by CombiPlate. This makes the colors unavailable to CombiPlate. See “Checking And Fixing The Color Map.,” page 336.

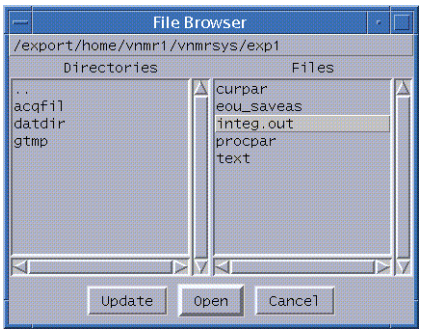
Analyzing VNMR Generated DATA

1. From a shell tool or terminal window UNIX, enter:

   `combiplate&`



2. **Click** on the **Load** button. An **Input screen** window appears with two input file options, **From VNMR** and **Reload**. Click on **From VNMR** to open the **File Browser**.



3. **Click** on the VNMR file containing the output from the macro *nlivast* such as *integ.out*. **Click** the **open** button to load the file. The *Update* button updates the browser.

4. The next *Input screen* opens, see Figure 94. Before the selected input file can be loaded, information on the number of row and the starting and ending columns must be provided.

The default values assume that the microtiter plate is in a portrait orientation and the cell in the upper left is H1 with columns labeled H->A and rows labeled 1->12 (top to bottom). If the number of rows and columns in the data set are different (fewer then 8 columns or 12 rows) enter the correct values in the appropriate fields. Remember to press the return key after each entry or the change will not take affect.

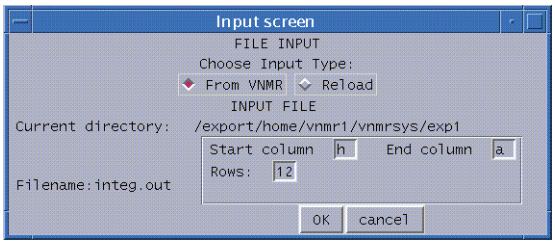


Figure 94. CombiPlate Column and Row Input Screen

5. **Click** the **OK** button to load the file and the row and column data into CombiPlate.
6. The File is loaded. The integral regions defined in step 6 of "Preparing VNMR Data For Analysis Using CombiPlate," page 331 are displayed in the scrolling text box at the at the bottom of the CombiPlate window in the scroll box, see Figure 91.
7. **Enter** the number of the integral region in the field entry box that you want associated with this color, see Figure 95. CombiPlate *does not* automatically assign

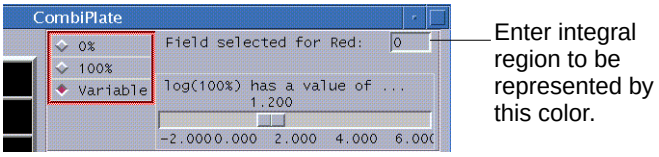


Figure 95. CombiPlate Field Box

a region to a color. Each integral region or field must be associated with a color. You must press the ENTER key after a value is placed in each field.

8. **Click** the **Display** button to load the data into the CombiPlate window. Depending upon the values of the integrals you may or may not have any colors displayed.
   - If no colors are displayed set all the *log(100% color intensity)* slider bars to (log(100%)= - 2.000) and **Click** the **Display** button.
9. Set the color intensity for each region.

   Color intensity for each region is controlled independently. Each region has two preset values, 0% and 100% and a variable. If the variable option is selected the intensity of the color can be related to the integral area.

   - If the *log(100% color intensity)* equals the largest integral value the color intensity is related to the integral area.
   - If *log(100% color intensity)* is set to a value equal to or less then the smallest positive non zero integral value for that region the intensity is not related to integral area. The color is binary, on or off. In this case the color is turned on (a non zero positive integral) or it is off (integral is either zero of negative).

   a. Set all the *log(100% color intensity)* slider bars to (log(100%)= - 2.000). This will set up a binary condition. The display in Figure 92 is set up to turn on the color of a given region only if there is a positive integral for that region.

   b. **Click** on the **Variable** radio button.

   c. **Click** the **Display** button.

d. To use the intensity of the color as a measure of the integral area of a region, move the slider bar for that regions to the right. **Click** on the **Display** button. If all the color for that region diappears, you moved the bar to far - move it back to the left. Select a value that provides a display with both the smallest integral value and largest integral value represented. Repeat for each region as desired. The display does not automatically update as the slider bar is moved. You must **Click** on the **Display** button to view all changes.

10. Click on any cell to view the integration data.

Analyzing CombiPlate DATA

1. From a shell tool or terminal window UNIX, enter:
   `combiplate`
2. **Click** on the **Load** button. An **Input screen** window appears with two input file options, **From VNMR** and **Reload**. Click on **From VNMR** to open the **File Browser**.
3. **Click** on a CombiPlate display file. **Click** the **open** button to load the file. The *Update* button updates the browser.
4. The next *Input screen* opens, see Figure 94. **Click** the **OK** button to load the file.
5. Continue by following the instructions in "Analyzing VNMR Generated DATA," page 334 from step 6 to the end.

Checking And Fixing The Color Map.

When you start CombiPlate (see Figure 96) the plate that is displayed is filled with synthetic data. This will give it an evenly graded color scheme running from green in the top left to yellow (top right), light blue (bottom left) and white (bottom right).

CombiPlate
h1 g1 f1 e1 d1 c1 b1 a1
h2 g2 f2 e2 d2 c2 b2 a2
h3 g3 f3 e3 d3 c3 b3 a3
h4 g4 f4 e4 d4 c4 b4 a4
h5 g5 f5 e5 d5 c5 b5 a5
h6 g6 f6 e6 d6 c6 b6 a6
h7 g7 f7 e7 d7 c7 b7 a7
h8 g8 f8 e8 d8 c8 b8 a8
h9 g9 f9 e9 d9 c9 b9 a9
h10 g10 f10 e10 d10 c10 b10 a10
h11 g11 f11 e11 d11 c11 b11 a11
h12 g12 f12 e12 d12 c12 b12 a12
0%
100%
Variable
Field selected for Red: 0
log(100%) has a value of ...
1.200
-2.000 0.000 2.000 4.000 6.000
Field selected for Green: 0
Field selected for Blue: 0
Current cell
Weight - Red Green Blue
Raw Data
Load... Save... Display Clear Help Exit

Figure 96. CombiPlate Window for VAST Data Analysis

If the color gradation is *not smooth*, you have run out of color map entries. The color coding of the results may be displayed incorrectly in the *CombiPlate* window. A warning will be issued whenever *CombiPlate* thinks that it has run out of colors. This warning is in red in the text window.

1. Fixing the color map.
2. Close any applications that may be color intensive such as:
   - Acrobat Reader
   - Netscape
   - Frame Maker
   - VNMR

   Applications running on the SUN computer all use colors form the color map and these colors are not available to CombiPlate. As a result, displays dependent upon gradations in color may be incorrect if a color is not available.
3. Exit *CombiPlate*
4. Restart *CombiPlate*.

   The desired color scheme should now be displayed.
5. If VNMR is running and you run out of colors, you may wish to save results inspection later when VNMR is not active.

10.5 Vast Process, Display, and Plot Macros

`combiplate` **View a color map for visual analysis of VAST microtiter plate (U)**

Syntax: (From UNIX) `combiplate`

Description: Opens the CombiPlate window, which provides a map of microtiter plate, allowing data to be viewed from individual sample wells. The window enables viewing integral region intensities by colors and color densities.

`combishow` **Display regions as red, green, and blue in CombiPlate window (M)**

Syntax: `combishow(r,g,b)`

Description: Displays integral regions shown on the spectrum as red (`r`), green (`g`), and blue (`b`) in the CombiPlate window. CombiPlate reads the regions automatically. 1, 2, or 3 integral regions can be designated. At least one integral region must be specified. Combishow displays spectra associated with individual wells.

`dsvast` **Display VAST data in a stacked 1D-NMR matrix format (M)**

Applicability: Systems with the VAST accessory.

Syntax: `dsvast<(display order,number of columns displayed)>`

Description: dsvast will arrange and display the traces from a reconstructed 2D data set (see (see vastglue) as an array of 1D spectra in a matrix of 1D spectra. If no arguments are provided, the number of rows and columns will be determined by the periodicity of the display order based on the `doneQ`. For example, if a block of 96 spectra (typical for a microtiter-plate) have been acquired using VAST

automation, the spectra will be displayed in a matrix 8 rows and 12 columns with the well label using the format (A->H)(1->12).

The spectra can be plotted using the macro `plvast`.

Arguments: `display order` is optional and its default value is the glue order as listed in `glueorderarray`. A `display order` can be defined using the `plate_glue` program.

`number of columns displayed`. The default value of is deduced by examining the periodicity of the requested display order. The `number of columns displayed` can entered as the second argument or as the first argument if the default `display order` is used.

`dsvast2d` Display VAST data in a pseudo-2D format (M)

Applicability: Systems with the VAST accessory.

Syntax: `dsvast2d(number)`

Description: If an array of 1D spectra have been acquired (in particular if a block of 96 spectra has been acquired using VAST automation, especially in a microtiter-plate format), and if these spectra have been glued into a reconstructed 2D data set (see `vastglue`), this macro will arrange and display them (on the screen) in a convenient pseudo-2D format (almost like an LC-NMR chromatogram).Well labels are not attached to the spectra and spectra are plotted with 8 spectra per row.

Arguments: The default is to display all the spectra (from 1 through `arraydim`) with 8 columns (spectra) and 12 rows. An optional argument `dsvast2d(number)` allows one to specify that only spectra from *1* through *number* should be plotted. The number of spectra displayed is rounded up to the nearest multiple of 8.

`nlivast` Produces a text file of integral regions without a sum region (M)

Applicability: Systems with VAST accessory.

Syntax: `nlivast(last)`

Description: Using predefined integral regions from the spectra for each well, `nlivast` writes a text file, integ.out, containing the integrals of the regions. The file is written into the current experiment. Does not add an additional region that is the sum of all the defined regions for each well (see dlivast).

Arguments: `last` is the number of the last well. The default is 96.

`nlivast2` Produces a text file with normalized integral regions (M)

Applicability: Systems with VAST accessory.

Syntax: `nlivast(well#)`

Description: Using predefined integral regions from the spectra for each well, `nlivast2` writes a text file, integ.out, containing the integrals of the regions. The file is written into the current experiment. Integrals are normalized to the integral specified by the argument `well.`The macro `nlivast2` does not add an additional region that is the sum of all the defined regions for each well (see dlivast). All of the spectra are integrated.

Arguments: `well` is the number of the reference sample well. The default reference is well 96.

nlivast3 **Produces a text file with normalized integral regions (M)**

Applicability: Systems with VAST accessory.

Syntax: `nlivast(well#)`

Description: Using predefined integral regions from the spectra for each well, `nlivast3` writes a text file, integ.out, containing the integrals of the regions. The file is written into the current experiment. Integrals are referenced to the integral specified by the argument `well`. The integral of spectrum from the sample specified by `well` is set to 1000. The macro `nlivast3` does not add an additional region that is the sum of all the defined regions for each well (see dlivast). All of the spectra are integrated.

Arguments: `well` is the number of the reference sample well. Reference integral set to 1000. The default reference is well 96.

plate_glue **Define a glue order for plotting and display (U)**

Applicability: Systems with VAST accessory

Syntax: `plate_glue`

Description: In a Unix terminal or shell window type plate_glue. The glue order is determined by clicking on the wells to be displayed. Save the glue order file in the user's vnmrsys/templates/glue directory.

plvast **Plot VAST data in a stacked 1D-NMR matrix format (M)**

Applicability: Systems with the VAST accessory.

Syntax: `plvast<(display order,number of columns plotted)>`

Description: plvast will arrange and plot the traces from a reconstructed 2D data set (see `vastglue`)as an array of 1D spectra in a convenient format (as a matrix of 1D spectra). If no arguments are provided, the number of rows and columns will be determined by the periodicity of the display order. For example, if a block of 96 spectra, as is typical for a microtiter-plate, have been acquired using VAST automation, the spectra will be plotted in a matrix 8 rows and 12 columns.

The default is to plot the spectra from 1 through arraydim (the number of spectra in the 2D data set). An optional argument (`plvast(##)`) allows one to specify that only spectra from 1 through ## should be plotted.

Arguments: `display order` is optional and its default value is the glue order as listed in `glueorderarray`.

`number of columns plotted`. The default value of is deduced by examining the periodicity of the requested display order. The `number of columns plotted` can entered as the second argument or as the first argument if the default `display order` is used.

plvast2d **Plot VAST data in a stacked pseudo-2D format (M)**

Applicability: Systems with the VAST accessory.

Syntax: `plvast2d<(number)>`

Description: If an array of 1D spectra have been acquired (in particular if a block of 96 spectra has been acquired using VAST automation, especially in a microtiter-plate format) and if these spectra have been glued into a reconstructed 2D data set (see vastglue), `plvast2d` will arrange and plot them (on the plotter) in a convenient pseudo-2D format (almost like an LC-NMR chromatogram). Well

labels are not attached to the spectra and spectra are plotted with 12 spectra per row.

Arguments: `number` specifies that only spectra from 1 through `number` should be plotted. The default is to plot all the spectra (from 1 through arraydim).

`vastget` Selects and displays VAST spectra (M)

Applicability: Systems with VAST accessory.

Syntax: `vastget(<well>,<well>, ...)`

Description: Selects and displays the spectra from any arbitrary well or wells using the well label(s) as arguments. The spectra are displayed in a `dss` stacked plot.

Arguments: `well` is the well label from which you want to select and display spectra. The wells are labeled [A->H][1-8].

`vastglue` Assemble related 1D data sets into a 2D (or pseudo-2D) data set (M)

Applicability: Systems with the VAST accessory.

Syntax: `vastglue(<rack,<zone>)`

`vastglue(<glue order>,<plate>)`

Description: Used to artificially reconstruct a 2D data set from a series of 1D data sets having similar filenames. It is crucial to ensure that the format of the file names of each of the 1D data sets is identical. `vastglue` reads in each 1D file, in succession, and adds it to the previous data, but in a 2D format. It assumes that file names are of the format obtained when using the default setting of autoname (`autoname=''`). If autoname has been redefined, use a macro like `vastglue2`. Save the resulting reconstructed 2D data set in the normal manner using `svf`.

Arguments: `rack` is the rack number; the default is 1. If you enter a `rack` number, you must also enter a `zone` number.

`zone` is the zone number; the default is 1. If you want to specify a `zone` number, you must enter a `rack` number.

`glue order` is the specific glue order to be defined based on the order defined in a `plate_glue` file. If `glue order` is specified, you can provide a `plate` number as the second argument and used with the `glue order` argument.

`vastglue2` Assemble related 1D data sets into a 2D (or pseudo-2D) data set (M)

Applicability: Systems with the VAST accessory

Syntax: `vastglue2<(number)>`

Description: Used to artificially reconstruct a 2D data set from a series of 1D data sets having similar filenames. It is crucial to ensure that the format of the file names of each of the 1D data sets is identical. `vastglue2` reads in each 1D file, in succession, and adds it to the previous data, but in a 2D format. It assumes that file names are of the format obtained using a non-default setting of `autoname` (`autoname='filename_R%RACK:%_Z%ZONE:%_S%SAMPLE#:%_'`). This definition must be hard coded into the macro by the user. If `autoname` has not been redefined, use a macro like `vastglue`. Save the resulting reconstructed 2D data set in the normal manner using `svf`.

Arguments: `number` is used to specify that only spectra from 1 through `number` are to be glued. The default is to glue all the spectra stored in the current directory that have the proper file name format (from 1 through `arraydim`).

10.6 Preparing the Hardware and Configuring VNMR

This section describes how to prepare the VAST hardware and configure VNMR for VAST operation. Use the procedures in this section if the Liquid Handler is being installed for the first time or if the Liquid Handler is being reconnected after it has been moved.

- Connecting the Transfer Tube
- Connecting the Air Tubing
- Connecting Signal and Power Cables
- Configuring VNMR for VAST

Connecting the Transfer Tube

This procedure describes how to connect the transfer tube between probe IN port and the injector valve (Gilson 819).

1. Determine the length of the transfer tube (between injector valve and probe), and then calculate the volume for this length using:

   $$V = \pi r^2 l = \frac{\pi}{4} D^2 l$$

   where V is the volume in cubic inches, r is the radius of the tube in inches, l is the length in inches, and D is the inside diameter (I.D.) in inches. To convert the volume from cubic inches to µL, multiply V by 16387. Some examples are shown below.

Length	*0.010-in. I.D.(Blue)*	*0.020-in. I.D.(Red)*
5 ft	77 µL	308 µL
10 ft	154 µL	616 µL
20 ft	310 µL	1240 µL

2. Connect one end of the transfer tube (blue PEEK tubing) to the IN port on the probe.
3. Use a union to connect the other end of the transfer tube to the stainless steel tube coming out of port 5 of the Rheodyne injector valve.

   Figure 97 shows the connection and part numbers for each part.

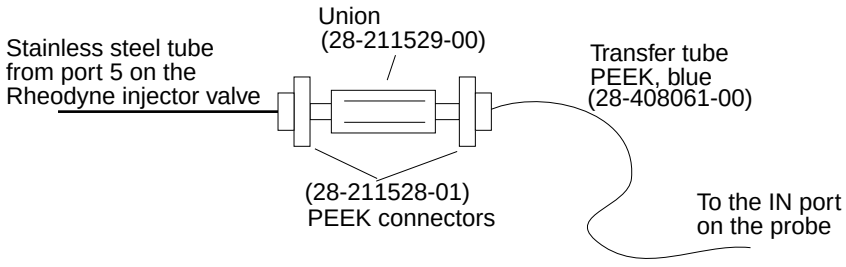


Figure 97. Connection Between the Rheodyne Injector Valve and Transfer Tube

4. Place the inlet tube of the Liquid Handler (with filter on the end) into the solvent container.

   The total volume from the injector port to the probe may be reduced by making a direct connection between the probe transfer tubing (going to the IN port on the NMR probe) and the injector port. This will bypass the Rheodyne injector valve.

Connecting the Air Tubing

This procedure describes how to connect the air tubing between the air regulator, the Valco valve, and the OUT port on the Microflow probe.

1. If the Microflow probe is not installed, install it now as described in the manual *Microflow NMR Probes Installation.*
2. Connect the Tygon (HDPE) tubing between an air regulator and port 3 of the Valco valve, as shown in Figure 98.
3. Use a PEEK connector to connect Teflon tubing between OUT port on the probe and port 2 of the Valco valve.
4. Make sure port 1 is open and insert a plug into port 4. Ports 5 and 6 are not used.

Connecting Signal and Power Cables

This procedure describes how to connect the signal and power cables.

1. Make sure the GSIOC cable is connected between the GSIOC connector on the back of the injector valve and the GSIOC connector on the back of the Liquid Handler. Refer to Figure 99.
2. Make sure the Remote Switching cable is connected. Connect one end of the cable 01-905126-00 to the Remote Switching cable on the back of the Valco valve. Connect the other end to the OUTPUT connector on the back of the Liquid Handler, as follows:
   a. On the OUTPUT connector, locate relay 2, which is the third set of two pins from the top.
   b. Connect the red wire to the top pin.
   c. Connect the white wire to the bottom pin.
3. Connect the RS-232 cable between the RS-232 connector on the back of the Liquid Handler and serial port A or B on the back of the Sun computer.
4. On the back of the Liquid Handler (below the RS-232 connector), verify that SW1 is set to 2 and SW2 is set to 6, as shown in Figure 99.
5. On the back of the injector valve (Gilson 819), verify that UNIT ID is set to 9, as shown in Figure 99.
6. Make sure the power is off in each of the following units:
   - Liquid Handler
   - Valco valve
   - Injector valve

   Connect the power cord for each unit into the power strip (with the power switch off), and connect the power strip to a power outlet.
7. Turn on the power to the units:

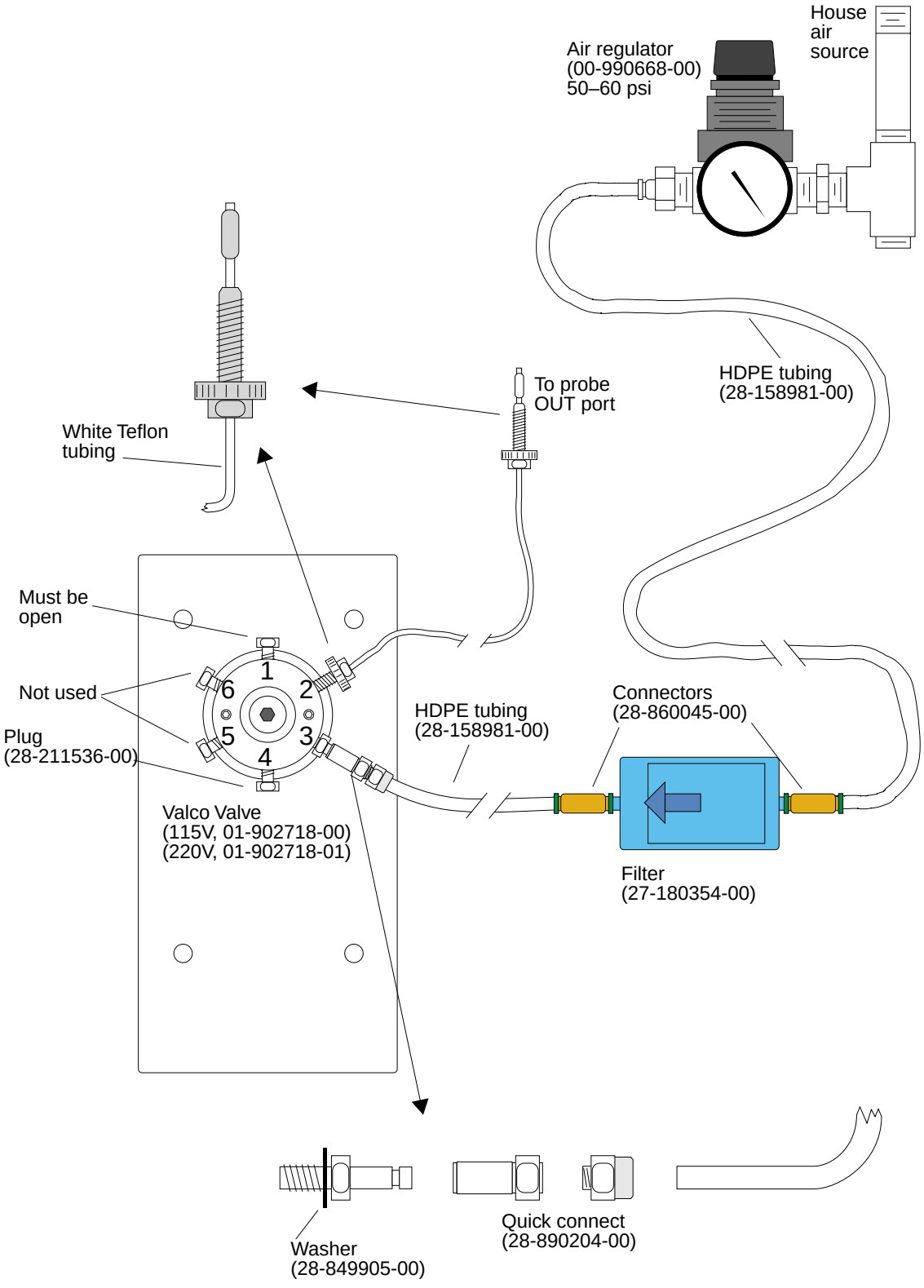


Figure 98. VAST Air Connections

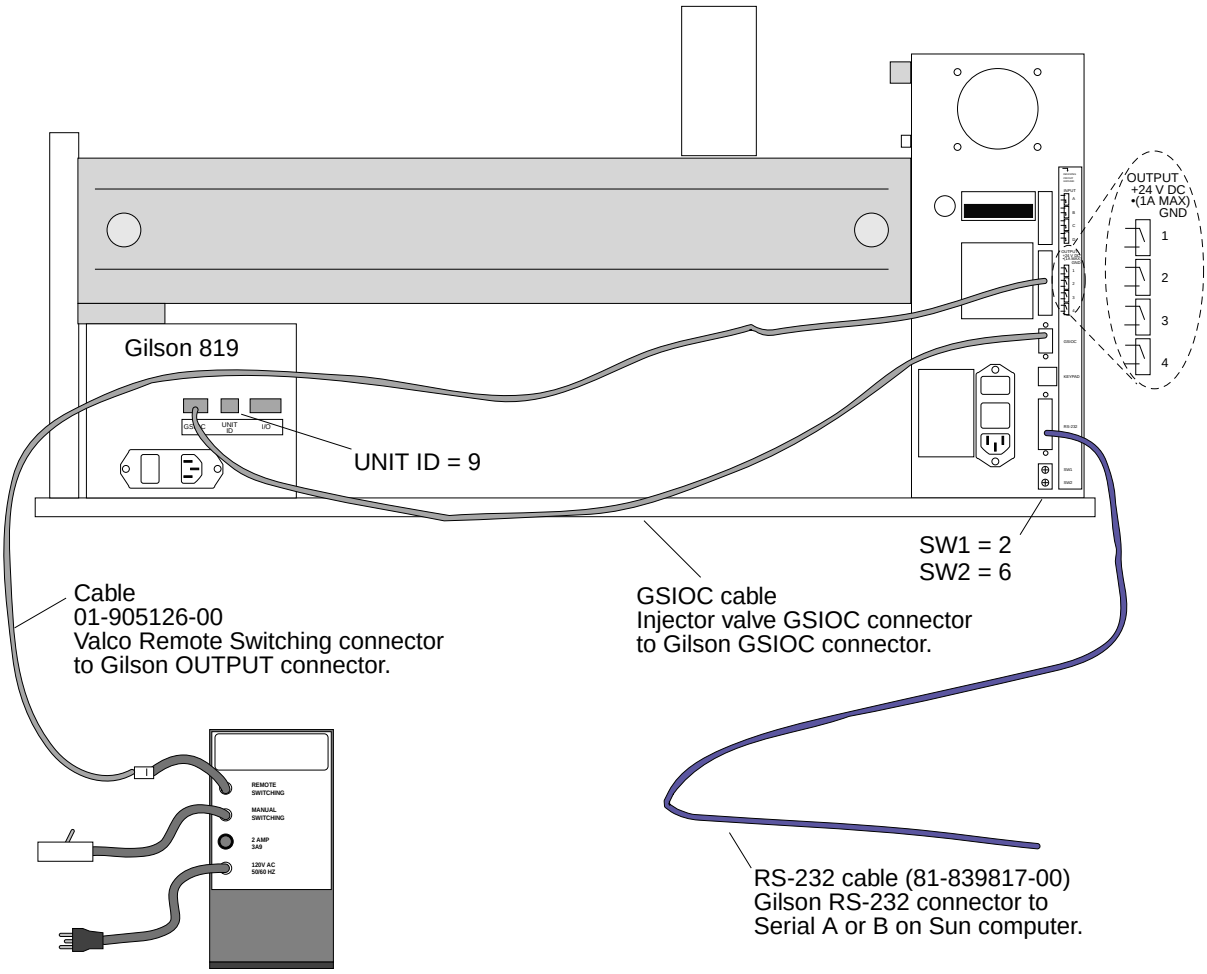


Figure 99. VAST Signal Cable Connections

- Power strip
- Liquid Handler
- Valco valve
- Injector valve

Configuring VNMR for VAST

This procedure describes how to use the VNMR configuration window to configure VNMR for the VAST accessory.

1. Log into VNMR as `vnmr1`.
2. Enter **`config`** in the VNMR input window.
3. Set the following values in the VNMR Configuration window:
   - Set **Sample Changer** to **VAST**.
   - Set **Serial Port** to the Sun computer serial port (**A** or **B**) that is connected to the Liquid Handler.

4. Click **Exit and Save** in the VNMR Configuration window.
5. Enter **`admintool`** and verify the settings for the serial port used with the Liquid Handler. Make sure `Template` is set to `Terminal-Hardwired` and that `Bidirectional` *is not* set under `Options`, which is only shown if `Detail` is set to `More` or `Expert`.

   To make changes to the serial port configuration, log in as `root` and use `admintool` to make the appropriate changes.
6. In the VNMR menus, select **Main–>Setup–>LCNMR–>Initialize VAST**.

10.7 Calibrating Volumes and Flow Rates

This section provides procedures for calibrating the following parameters for VAST accessory operation:

- Probe Volume
- Sample Volume
- Probe Slow Rate or Probe Slow Rate and Probe Slow Vol
- Calibrate needle rinse and Z position

Also in this section are some guidelines for calibrating the XYZ positions for the Liquid Handler arm.

To Calibrate Probe Volume

Probe Volume is the total volume of fluid contained in the transfer tube and the probe. You calibrate Probe Volume to determine the optimum amount of fluid necessary to adequately fill the flow cell of the Microflow probe. Figure 100 illustrates where the different volumes are in a VAST system.

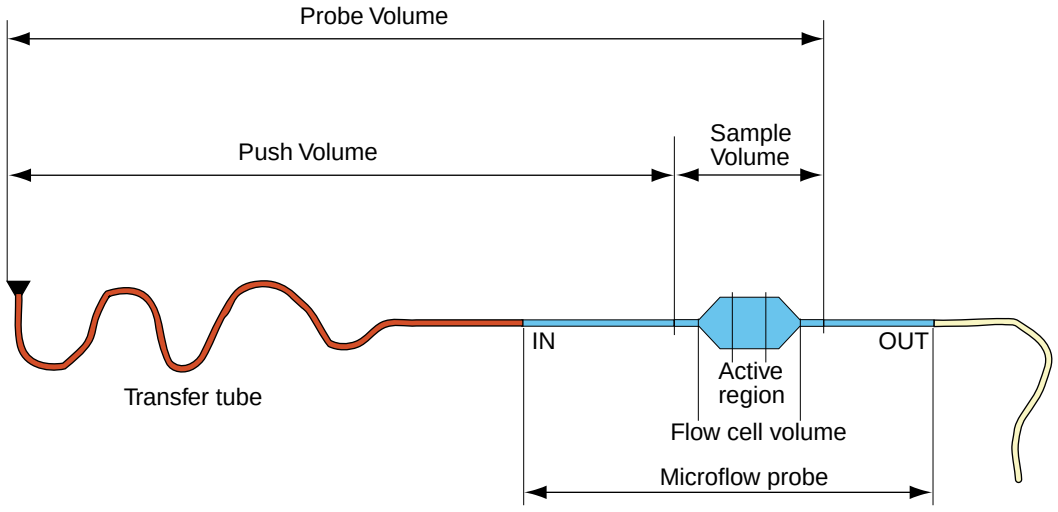


Figure 100. Microflow Probe and Transfer Tube Volumes

Calibrating Probe Volume requires a comparison of several data sets to one of two control data sets. The first control data set is acquired after using a syringe to completely fill the Microflow probe with D_2O. The second control data sets is acquired after filling the probe

with the Liquid Handler (with either D_2O or a prepared sample) by setting the Probe Volume parameter larger than needed. Finally, several data sets are acquired (with either D_2O or a prepared sample), each with a slightly smaller value of Probe Volume, until the optimal probe volume is found.

One of two methods can be used to find the optimal Probe Volume:

- *Using D_2O* – For Varian installation engineers and users who do not need to have Sample Volume calibrated. This method uses D_2O to minimize chemical handling, but D_2O does not allow you to do the Sample Volume calibration.
- *Using a prepared sample* – For users who want to calibrate Sample Volume. This method, which is described in "To Calibrate Sample Volume," page 350, requires preparing a sample, such as 1% CH_3CN or CH_3OH in D_2O.

Use the following procedures to calibrate the Probe Volume parameter:

- "Obtaining a Control Data Set Using a Syringe," next
- "Verifying the Control Data Sets Using the Liquid Handler," page 347
- "Finding the Optimum Value of the Probe Volume Parameter," page 349

Start with a sample that provides an observable ^1H NMR signal. The default is D_2O. The sample should not contain any dissolved or undissolved solids.

Obtaining a Control Data Set Using a Syringe

You obtain the first data set after using the syringe to completely fill the Microflow probe with D_2O.

1. Enter **`gilson`** to open the Liquid Handler window. Select the **Main Control** pane and make sure **Air valve** is set to **OFF**.
2. Disconnect the high-pressure air valve from the Microflow probe OUT connector. Connect a waste line to the probe OUT connector. See Figure 101.
3. Use the syringe and manually fill the Microflow probe with D_2O. Inject the D_2O until it begins to drip out of the waste line.

   Refer to the manual *NMR Probes Installation* for details on filling and flushing the Microflow probe.
4. After the probe is full:
   - Tune the probe
   - Lock and shim
   - Acquire an NMR spectrum

   Use a parameter set appropriate for determining `pw90` on the Microflow probe. Use a 90° pulse and a sufficiently long `d1` delay.
5. Expand around the resulting water resonance (about a 100-Hz window) and adjust the integral reset regions to obtain an integral of the residual HOD resonance.

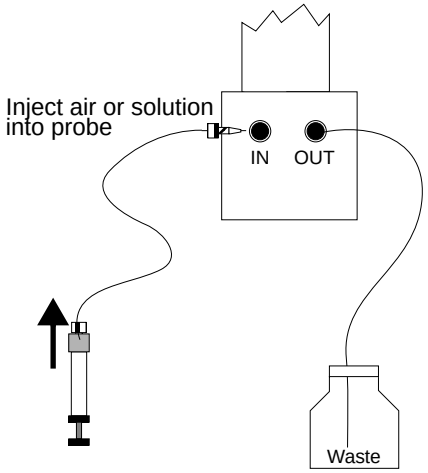


Figure 101. Injecting Solution into a Microflow Probe

6. Save the file and write down the lock level.

   You will eventually compare the resolution, lineshape, peak height, peak area, and lock level of future data sets to this data set.

7. Using the syringe, flush the probe with air to remove the D_2O solution.

   Refer to the manual *NMR Probes Installation* for instructions on flushing the Microflow probe.

8. Disconnect the waste line from the probe OUT port and reconnect the air line.

Verifying the Control Data Sets Using the Liquid Handler

With this procedure, you use the Liquid Handler to verify the control data set. You can use either D_2O or a prepared sample.

Using D_2O – Read these points and go to step 1 below:

- Change the rinse solvent for the Liquids Handler to the same D_2O used above. Currently, the Liquid Handler is supplied with a waste container for the needle rinse station, but it is not supplied with any additional solvent reservoir containers.
- With this method, you use the same D_2O solution used for both the control data sets and the Probe Volume calibration (and for the rinse solvent).

Using a Prepared Sample – Read these points and go to step 1 below:

- With this method, you prepare a sample of your choice and fill a container in a Liquid Handler rack (instead of using just the rinse solvent). Although this requires the end user to make up a sample, such a sample is also required for the Sample Volume calibration, described in the procedure "To Calibrate Sample Volume," page 350.
- The test is easiest if the ^1H spectrum of the sample contains an isolated, tall, sharp resonance; a suggested sample is 1% CH_3CN or CH_3OH in D_2O (with D_2O as the rinse solvent), although almost any sample with a sufficiently strong signal (signal-to-noise greater than 100:1) can be used.
- Do not try to run the test by observing the HOD line of D_2O samples stored in open containers because such samples absorb too much H_2O from the atmosphere during the test to allow accurate peak areas to be measured.

1. Place the Liquid Handler inlet line (fritted) into the supplied bottle of D_2O.

   Cover the opening of the D_2O bottle to prevent the absorption of H_2O.

2. If you are using a prepared sample, do the following substeps. If you are using D_2O, skip to step 3:

   a. Prepare your sample and transfer it to the sample wells in a Liquid Handler sample rack.

   b. Make sure each sample well contains enough sample to run the calibration. The total volume should be more than Probe Volume. Remember to write down the locations where you place the samples; you will use these locations in later steps.

3. Flush the Microflow probe with air:

   a. Enter **`gilson`** in the VNMR input window to open the Liquid Handler window.

   b. Click on the **Main Control** pane.

   c. Set **Air valve** to **ON**.

d. Wait 1 to 2 minutes to ensure that the Microflow probe is empty.

e. Set **Air valve** to **OFF**.

4. Click on **Prime pump** in the **Main Control** pane of the Liquid Handler window. Wait for the priming routine to finish.

5. Switch to the **SAMPLE Def.** pane and set the following values:
   - **Probe Slow Rate** to **0.3**
   - **Probe Fast Rate** to **0.3**
   - **Probe Slow Vol** to **250**
   - **Number of Rinses** to **0**
   - **Keep Sample** to **No**

6. Set **Probe Volume** to a value large enough to ensure that the active region of the Microflow probe is completely filled. Use one of the following values as a starting point.
   - More than 440 µL for a 60-µL probe
   - More than 600 µL for a 120-µL probe

   The value needed also depends upon the length and diameter of the transfer tube that connects the Microflow probe IN port to the Liquid Handler inject port. The default transfer tubing is 0.010-in. I.D. and 10-ft. long.

   This value must also be constrained to be somewhat smaller than the available syringe volume (Sample Volume must be less than SyringeVolume *minus* the absolute value of Sample Extra Vol *plus* Retrieve Extra Vol).

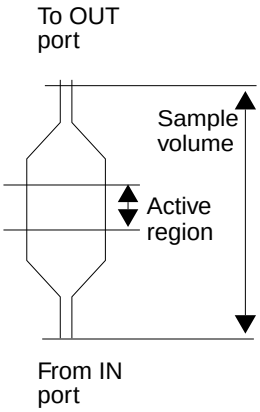


Figure 102. Microflow Probe Flow Cell

7. Set the **Sample Volume** value as follows:
   - If you are using D_2O, set Sample Volume to 0. This value ensures that all of the fluid will come from the solvent bottle and not from the sample containers on the racks.
   - If you are using a prepared sample stored in a container in a rack, set Sample Volume to 150 µL for a 60-µL probe or to 250 µL for a 120-µL probe.

8. Click the **Save Now** button in the **SAMPLE Def.** pane.

9. Make sure all racks and vials are properly placed on the bed of the Liquid Handler so that the robotic arm will not accidently get caught on anything.

WARNING: **Avoid personal injury by using caution around the Liquid Handler work area. The Liquid Handler is capable of operating at high speeds. Leaning into the Liquid Handler work area, placing your hand or arm in its path, or wandering within range of the arm movements could result in serious physical injury.**

CAUTION: **Avoid damage to the Liquid Handler and other equipment by clearing the work area around the Liquid Handler. The Liquid Handler arm moves rapidly around its work area.**

10. Enter the `loc` parameter as follows:

- If you are using D_2O, enter **loc=1 change**. Note that the location used is unimportant since all the fluid comes from the rinse solvent container.
- If you are using a prepared sample stored in a container in a rack, enter **loc=# change**, where # is the location of the prepared sample.

Verify that the sample changed properly. If so, go to the next step.

11. Enter **ga**.
12. Compare the resulting spectrum to the control spectrum.

    The spectrum you just obtained should be as good as the first control spectrum in all aspects (lock level, resolution, lineshape, peak height, and peak area/integral).

    - If the peak height and peak area differ, but the lineshape and lock level are as good, it could be because of a different amount of H_2O absorbed from the atmosphere—if so, save this spectrum as control #2 for future comparisons.
    - If the resolution is worse, or especially if the lock level is lower, either the current Probe Volume is not large enough to fill the NMR probe or something else is wrong that needs to be corrected before continuing.
13. If the two spectra compare favorably and you have saved the second spectrum, enter: **loc=0 change**.
14. After the sample change finishes, manually turn on the air valve to ensure that the Microflow probe is completely empty before continuing with the rest of this procedure.

    If part of the sample is not being recovered (for example, if Probe Fast Rate or Probe Slow Rate is too large), you can still perform the Probe Volume calibration but you need to perform the air-valve routine between each spectrum to blow the probe empty.

Finding the Optimum Value of the Probe Volume Parameter

The ideal Probe Volume value should be just slightly bigger than the minimum required volume (adding 10 to 20 µL of extra volume) to ensure reliable filling during routine operation. Write down this optimized Probe Volume value should for future use.

Because the value obtained depends upon the probe and the transfer tubing being used, both of these variables should be documented. Table 49 lists typical Probe Volume values for various probe and transfer tube combinations.

Table 49. Approximate Probe Volume Values for the Various Probes and Transfer Tubes

Microflow Probe Flow Cell Volume, Tube Diameter	*Probe Volume with 10-ft 0.01-in. ID transfer line*	*Probe Volume with5.5-ft 0.01-in. ID transfer line*
60 µL, 0.005/0.01 in.	410 µL	320 (+20 µL)
60 µL, 0.01/0.02 in.	440 µL	350 µL
120 µL, 0.01/0.02 in.	520 µL	450 µL
240 µL, 0.01/0.02 in.	820 µL	750 µL

Note: The dead volume of a 10-ft length of 0.010-in. tubing is approximately 155 µL.

To find the optimum probe volume:

1. Acquire a series of spectra (on different sample locations) using different values of the Probe Volume parameter.

Use at least 10 different values for Probe Volume, in 10 µL steps, decreasing from the Probe Volume value used in the previous procedure (this should be greater that the corresponding value in Table 49). Use the largest expected value of the Probe Volume and work towards smaller values. When you run spectra with different values of the Probe Volume, make sure the resulting value of the Push Volume remains positive.

Remember to click the **Save Now** button in the SAMPLE Def. pane after each change. It is always a good precaution to blow the probe empty once or twice during the series of spectra.

If the Flow Rate parameters have not been calibrated, the probe should be blown empty after each sample (the command `loc=0 change` can be used for this).

Invoke each change with `loc=# change`, where # is location of the prepared samples.

Do not shim between spectra. All measurements should be reproducible, ideally with minimal hysteresis, regardless of whether measurements range from maximum-to-minimum or minimum-to-maximum values.

2. Make a chart of the Probe Volume value versus the quality of the spectrum—lock level, resolution, lineshape, peak height, and peak area/integral.

   As long as the Probe Volume is large enough to fill the active region of the Microflow probe, the spectral quality will be good. If the spectral quality is not good, the Probe Volume is too small. If the active region is not filled, the spectral quality will be poor and will rapidly deteriorate as the Probe Volume gets smaller. Also, any samples in the future will be hard to shim.

3. Choose the smallest value of Probe Volume that still generates good data. Write down this Probe Volume value as well as the probe and transfer tube geometry (length and diameter) used.

4. After you find the optimal Probe Volume, use the same sample to calibrate Sample Volume as described in the next section “To Calibrate Sample Volume.”

To Calibrate Sample Volume

Sample Volume can be equal to Probe Volume, or it can be a portion of Probe Volume, where the remainder of Probe Volume is filled with Push Volume. In other words, the sum of Sample Volume and Push Volume equals Probe Volume. Portioning Probe Volume into Sample Volume and Push Volume allows you to use less sample because you fill the dead volume of the transfer tube with Push Volume. Using a gas bubble to push the sample is undesirable.

You calibrate Sample Volume to find the optimum (minimum) volume of sample to fill the flow cell of the Microflow probe. An accurate calibration of Sample Volume depends upon an accurate value of Probe Volume. You should verify or recalibrate Probe Volume immediately before calibrating Sample Volume.

As a general rule, the larger the value of Push Volume, and the “stickier” the solvent, the lower the NMR sensitivity—DMSO is “sticky,” D_2O is not; other organic solvents are intermediate. As another general rule, the bigger the difference in magnetic susceptibility between the sample and the rinse solvent, the bigger the chance that NMR resolution will suffer for a given Sample Volume. If the NMR resolution suffers when a push solvent is used, then you probably need to either increase Sample Volume or match the magnetic susceptibilities of the two solvents better; the line broadening effect usually cannot be corrected by shimming.

The Sample Volume calibration requires some visible difference in the ^{1}H NMR spectra between the sample and the rinse solvent. The sample needs to have some additional ^{1}H NMR resonances, which are not present in the rinse solvent. You can use 1% CH_3OH, CH_3CN in D_2O, or the same prepared sample used for calibrating Probe Volume. This calibration cannot be done with D_2O alone (see "To Calibrate Probe Volume," page 345).

Note: *Do not reshim during this calibration*. Any given set of conditions should produce the same spectral quality reproducibility—if not, it might be a good idea to enter `loc=0 change` and then blow the probe empty with the air valve between each run. If this is necessary, it suggests that the values of Probe Slow Rate or Probe Fast Rate are too large.

1. If not already done, prepare your sample and transfer it to the sample wells in a Liquid Handler sample rack.

   Make sure the sample wells contain sufficient amounts of the sample to accomplish the calibration.

2. Set the following values in the **SAMPLE Def.** pane of the **Liquid Handler** window:
   - **Sample Volume** to 150 uL for a 60 uL probe or to 250 uL for a 120 uL probe
   - **Keep Sample** to **No**
   - **Number of Rinses** to **0**
   - **Probe Volume** to the value calibrated by the procedure "To Calibrate Probe Volume," page 345.

3. Click the **Save Now** button in the **SAMPLE Def.** pane.

4. Obtain a spectrum on the first sample and expand around a representative sharp peak (other than HOD) in the ^{1}H NMR spectrum.

5. Save this data set and note the peak height (and lineshape).

6. Obtain additional spectra with decreasing values of Sample Volume (Probe Volume remains fixed) and look at the peaks heights (or S/N).

7. Plot the peak heights (or S/N) as a function of the Sample Volume.

   The NMR peak heights should decrease as the Sample Volume parameter decreases, even when the Probe Volume parameter is kept constant. (the NMR peak heights should drop to zero when Sample Volume is 0).

   The exact nature of the curve you plot depends upon the solvent being used (DMSO generates a curve, while D_2O produces more of an angle or *knee* at the intersection of two straight lines), but this chart allows you to determine how much sample must be used to obtain the percentage of the available signal-to-noise. See Figure 103.

   For a 60-uL probe in D_2O with Probe Volume set to 350, the signal intensity when Sample Volume is 150 should be 75 to 95% of the signal intensity obtained when Sample Volume is 350.

To Calibrate Flow Rate Parameters

This section describes how to calibrate the parameters Probe Slow Rate, Probe Slow Vol, and Probe Fast Rate.

- If these parameters are set too large, the sample is not completely removed from the probe and subsequent samples are significantly contaminated.
- If these values are set too small, time is wasted by running the system at a slower than optimal speed.

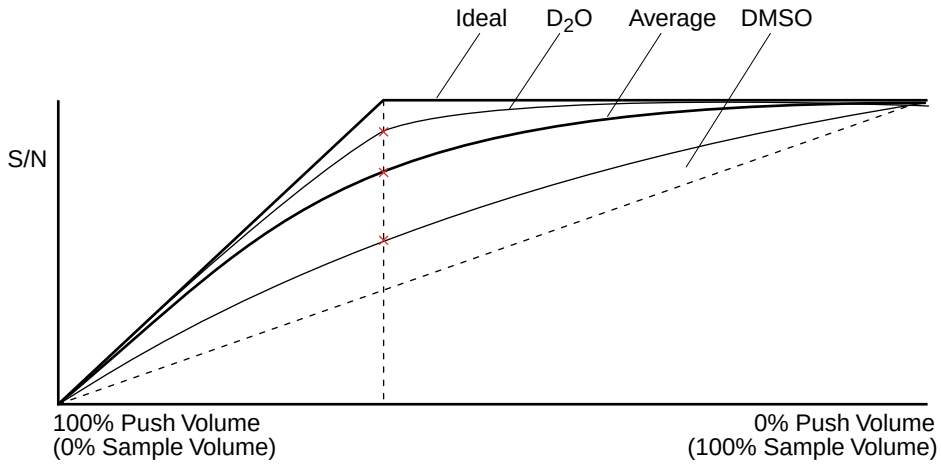


Figure 103. Finding the Optimum Sample Volume

The values obtained are *very dependent* upon the following:

- Sample solvent
- Rinse solvent
- Probe flow cell volume
- Tubing used, length, I.D., etc.
- Pressure of the gas delivered to the Valco valve.

Choose one of the three methods described below. In all three methods, Probe Slow Rate and Probe Fast Rate must both be small enough (giving slow enough flow rates) that the following are true:

- No leaks or drips occur at either the syringe vent port or the injector port when pushing
- No cavitation bubbles appear in the syringe when retrieving solvents (or in the Gilson tubing connecting the needle to the syringe)
- When Keep Sample is set to Yes, all of the sample is recovered

Method 1: Fast Setup, Slow Runs

This method consists of just setting the parameters to be so slow that the solvent will never leak or cavitate. This method is fast to set up (since no calibrations are done), but slow in operation (since no parameters are optimized). This method is not recommended for high-throughput operation, but may be acceptable for testing or open-access operation.

1. Set the following values in the **SAMPLE Def.** pane of the Liquid Handler window:
   - **Probe Slow Vol** equal to **Probe Volume or larger**.
   - **Probe Slow Rate** to **0.3**
   - **Probe Fast Rate** to **0.3**

   Larger values might be possible for your system, but 0.3 mL/min should work for the most viscous solvent in the most narrow tubing.

   If Probe Slow Vol equals Probe Volume, setting Probe Fast Rate has no effect, but you should set it to a small value just for safety.
2. Click the **Save Now** button.

Method 2: Calibrating Probe Slow Rate for Faster Runs

This method consists of performing a simple calibration to allow the Liquid Handler to run faster. This flow rate value must be determined for the most viscous sample in the system. This might be the rinse solvent, a push solvent, or a sample, but it must be the most viscous sample you expect to encounter!

1. Set the following values in the **SAMPLE Def.** pane of the Liquid Handler window:
   - **Probe Slow Vol** equal to **Probe Volume or large**r.
   - **Probe Slow Rate** to **0.3**
   - **Probe Fast Rate** to **0.3**
   - **Number of Rinses** to **1**
2. Click the **Save Now** button.
3. Run samples with the solvents in question repeatedly, increasing the Probe Slow Rate in 0.1 mL/min steps each time, while watching the clear glass syringe barrel on the Liquid Handler.

   If bubbles start appearing in the syringe barrel or needle tubing when the sample is being withdrawn from the probe, Probe Slow Rate is too large (the bubbles typically get bigger as the Probe Slow Rate value gets larger). Any cavitation bubbles formed must disappear *before* the needle (probe) is pulled up out of the injector port; otherwise, sample is left behind in the probe (all the bubbles always disappear the moment the needle [probe] is pulled out of the injector port).
4. Use the largest value of Probe Slow Rate that still allows complete sample recovery. Table 50 lists some typical flow rates for common solvents.

Table 50. Typical Flow Rates for Common Solvents

Solvent	*Typical Flow Rate**
CH_3CN	4.4mL/min for 0.01/0.02**
50:50 CH_3CN:D_2O	1.5 mL/min for 0.01/0.02**
D_2O	1.0 mL/min for 0.01/0.02** 0.7 mL/min for 0.005/0.01**
DMSO	0.5 mL/min for 0.01/0.02**

* Flow rates depend primarily upon the inner diameter of the inlet tubing.
** Probe inlet tubing diameter (in inches/outlet tubing diameter [in inches]).

Method 3: Calibrating Probe Slow Rate and Probe Slow Vol for Fastest Runs

This method consists of performing a more complex, three-parameter calibration. Although this calibration allows the system to run with the fastest possible throughput, it needs to be reoptimized for every different solvent and plumbing geometry. The nature of the calibration precludes a simple step-by-step description.

After the majority of a sample (or rinse) is withdrawn from the Microflow probe, the remainder can be withdrawn faster. The remainder is withdrawn at a speed determined by the Probe Fast Rate parameter. This calibration consists of balancing Probe Slow Vol and Probe Slow Rate with Probe Fast Rate.

A faster Probe Slow Rate or a smaller Probe Slow Vol necessitates a slower Probe Fast Rate.

The criteria for optimization are as follows:

- No cavitation bubbles in the syringe or needle tubing
- The full volume of recovered samples is returned to the original sample containers
- Sample insertion and removal occur as fast as reasonably possible
- Probe Slow Vol is greater than Sample Volume

To Calibrate XYZ Positions of the Arm

You have control over the XYZ coordinates of the rinse station, injector port, and sample racks on the Liquid Handler. You can make changes to these units through the Calibrations pane of the Liquid Handler window See “VAST Interface Description” on page 355.

The X and Y positions of all three units will probably never need recalibration after installation. You might possibly need to calibration the Z position for the following:

- *Rinse station* – You can adjust the Z position of the needle to place the needle up to 45 mm down inside the rinse station for an inside-and-outside needle rinse.See “To Calibrate XYZ Positions of the Arm” on page 354.
- *Injector port* – You might have to optimize the Z position after changing the needle to ensure a leak-free injection.
- *Sample racks* – No calibration of Z position needed.

10.8 Acquiring Data on Standard Test Samples

After the Probe Volume is calibrated, you can acquire data on some standard test samples to verify that all the VAST hardware and software are operational.

1. Fill four different sample locations with a standard sample.

   This test does not require a specific sample. You can use D_2O, the prepared sample, sucrose, or methyl p_hydroybenzoate.

2. Set the following values in the **SAMPLE Def.** pane of the Liquid Handler window:
   - Set **Probe Volume** to the value determined in the section “To Calibrate Probe Volume,” page 345.
   - **Sample Volume** to 150 µL for a 60-µL probe or to 250 µL for a 120-µL probe
   - **Keep Sample** to **Yes**
   - **Number of Rinses** to **1**
   - Make sure the **Push Volume** is positive
3. Click the **Save Now** button.
4. Acquire spectra both manually and with the `enter` program.
   a. Acquire spectra manually:
      `loc=# ga`

      where # is one of the four locations filled in step 1.
   b. Acquire the same spectra using the `enter` program to obtain ^1H NMR spectra of the four samples.

10.9 Evaluating Carryover

The amount of carryover from one sample to the next can never be zero in a flow probe. The amount of carryover is affected by the probe geometry, the direction(s) of solvent flow, the number of rinses, the effectiveness of sample recovery, and in particular by the solvents used. Multiple rinses reduce carryover, but the additional time consumed by more that one rinse is probably prohibitive.

To measure the amount of carryover:

1. Select a solute that generates an NMR singlet (e.g., CH_3OH, CH_3CN, TSP, or DSS) and make a concentrated solution of this solute (1 to 10%) in your solvent of choice (e.g., D_2O).
2. Place this sample in location 1, then place pure solvent in locations 2 and 3.
3. Set up proper parameters in the VNMR parameter set and in the Liquid Handler window.
4. Enter **`loc=1 change`**.
5. Enter **`ga`** to obtain a ^1H NMR spectrum. Expand the spectrum around the region of interest, and set the vertical scale, integral resets, and integral scale.
6. Save the data and plot the data:
   `svf(`*`filename`*`)`
   where *`filename`* is a name you choose (e.g., `svf('mydata')`).
   `region vp=12 pl dpir page`
7. Enter **`loc=2 ga`**.
8. Use the resulting spectrum to quantitate the amount of carryover remaining from sample 1.
9. Enter **`loc=3 ga`** to repeat the process for the sample in location 3.
   With one rinse, the carryover averages about 1%, but the value you obtain can be highly variable (0 to 8%) depending upon the solvents used and other specifics of the experiment.

10.10 VAST Interface Description

The user interface on the front panel of the Gilson 215 Liquid Handler is a display and a STOP button.

The VAST interface to VNMR includes the LC-NMR pane (shown in Figure 78), which provides controls for setting up VAST experiments and the gilson window and tabs shown in figures 30 through 33.

Otherwise, the VAST accessory is set up using the Liquid Handler window in VNMR, which provides the following four panes for setting up the VAST accessory:

- SAMPLE Def. (shown in Figure 104) – For defining sample volumes and fluid-control parameters.
- Rack Def. (shown in Figure 105) – For defining which racks and containers are available to the Liquid Handler.
- Main Control (shown in Figure 106) – For setting up and controlling the hardware.
- Calibrations (shown in Figure 107) – For calibrating arm positions.

Each of these panes is described in a separate section below. Table 48 lists the default values for the fields in the Liquid Handler window.

SAMPLE Def.

The Sample Def. pane (shown in Figure 104) contains most of the routinely used fluid-control parameters. These parameters allow you to

- Adjust sample volume
- Specify whether to keep the sample
- Perform probe rinses
- Specify flow rates

Clicking the **Save Now** button saves the values in the Liquid Handler panes and applies them to the next injection. Clicking the Additional Parameters button expands the screen and provides more fields, as shown in Figure 104.

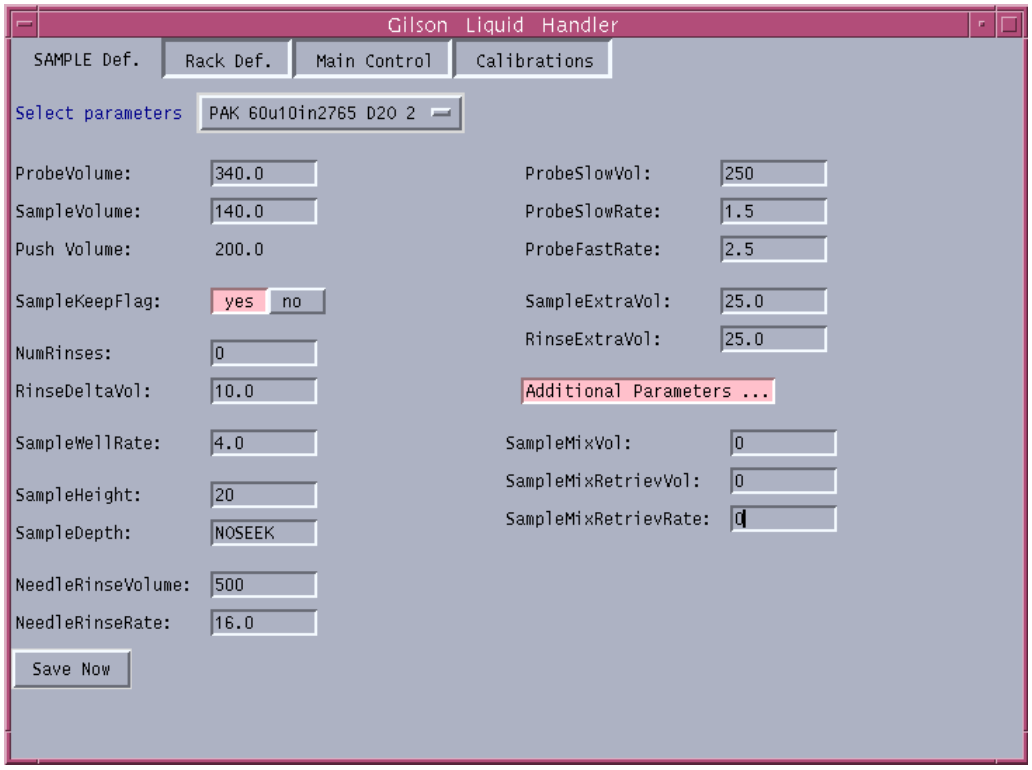


Figure 104. Sample Definition Window, Expanded View

After the values are set and saved in this window, all NMR activities (typically controlled by either the `enter` program or the `loc` command) will use the same values for every sample, until the parameters in the Liquid Handler window are changed again.

The SAMPLE Def. window contains the following fields:

Select parameters	The file names of the available sets of Gilson parameters. The files are selected by a pull-down menu. The file name of an active file can be changed by double-clicking on the current name.

ProbeVolume	Total volume, in µL, held in the probe flow cell plus the volume held in the transfer tubing. In other words, this is the volume of liquid that the Liquid Handler will need to fill the probe flow cell, the dead volume in the tubing, etc. These values can vary widely, depending upon the length and inner diameter of the transfer tubing (see Table 49); however, typical values are as follows: • For a 60-µL Microflow probe, the default value of Probe Volume is about 440 µL (with 10 ft of 0.010-in. I.D. transfer tubing). • For a 120-µL Microflow probe, the default value of Probe Volume is about 520 µL.
SampleVolume	The amount of sample, in µL, needed to fill the probe flow cell. This is the amount that will be removed from the sample container. This value depends only on the Microflow probe that is used and not on the transfer tube. This volume can be calibrated to obtain the best NMR sensitivity per amount of sample injected. The default value is to set Sample Volume equal to Probe Volume; however, smaller sample quantities can be used at the expense of sensitivity. If this value is set too small, the NMR sensitivity, and possibly the NMR resolution, decreases. If this value is too large, you will consume more sample than is necessary. • For a 60-µL Microflow probe, a typical value of Sample Volume is about 140 µL. • For a 120-µL Microflow probe, a typical value of Sample Volume is about 240 µL
PushVolume	The amount of solvent, in µL, used to “push” the sample into the probe flow cell. Push Volume is automatically calculated Probe Volume minus Sample Volume). A push volume allows you to minimize the amount of sample used to create the Probe Volume.
SampleKeepFlag	Sets whether or not the sample is kept after NMR is finished. • Set to Yes to have samples returned to the vials on the sample racks. • Set to No to have samples placed in waste. The used sample is flushed into the rinse station container.
NumRinses	The number of NMR probe rinse cycles performed between sample changes. Typical values are either 0 or 1, where 1 is recommended. A needle rinse is performed between each sample change, but a probe rinse is only performed if a value is entered in the Number of Rinses field. More rinses (e.g., Number of Rinses set to 2 or more) reduces carryover, but takes longer.

	An NMR probe rinse cycle consists of the following actions: After the previous sample is aspirated from the probe and dispensed to waste or to the sample vial, the syringe aspirates from the solvent reservoir a volume equal to Probe Volume + Rinse Extra Vol. This volume is injected (dispensed) into the probe, and then immediately withdrawn from the probe and dispensed into the waste container.
RinseDeltaVol	The amount of solvent, in µL, in addition to the Probe Volume, injected into the probe during a rinse cycle. This volume ensures that the flow cell is thoroughly rinsed. A typical default is 10 µL; any value over 2 µL seems sufficient.
ProbeSlowVol	The volume, in µL, removed from the NMR probe at the Probe Slow Rate. An easy default is to set Probe Slow Vol equal to Probe Volume. • If Probe Slow Vol is too small, the sample might not be completely removed from the NMR probe. • If Probe Slow Vol is too large, turnaround times might be longer than necessary. After the Probe Slow Vol is removed, the rest of the Probe Volume is removed at the faster rate (Probe Fast Rate).
ProbeSlowRate	The flow rate, in mL/min., used to remove the initial volume specified by Probe Slow Vol. • If this rate is too fast, bubbles will appear in the syringe when the sample is withdrawn (because of the vacuum that is formed) and not all of the sample is recovered when the sample is removed from the probe. • If this rate is too slow, turnaround times might be longer than necessary. Values typically range from 0.5 for DMSO to 1.0 for D_2O to 1.5 for CH_3CN but depend greatly on the sample and hardware.
ProbeFastRate	The flow rate, in mL/min., used to inject samples into the NMR probe and to remove the remaining volume (after Probe Slow Vol) from the NMR probe. Some factors that might limit this speed are solvent composition (viscosity), NMR probe volume, and tubing diameter. • If the Probe Fast Rate is too fast, the pressure relief valve next to the syringe leaks when sample is pushed into the NMR probe, and bubbles can appear in the syringe when the sample is withdrawn (because of the vacuum that is formed). As a result, not all of the sample is recovered when the sample is removed from the probe. • If this rate is too slow, turnaround times might be longer than necessary.
SampleExtraVol	The extra amount of sample, in µL, removed from the probe when the sample is aspirated from the probe. This volume helps ensure that all the sample is recovered and that the probe is clear before the next sample is injected. A typical default is 25 µL; any value over 5 µL seems to be sufficient.

RinseExtraVol	The extra amount of solvent, in µL (in addition to Probe Volume and Rinse Delta Vol), that is removed from the probe during a rinse cycle. This volume ensures that more solvent is removed than injected, thus clearing the probe. A typical default is 25 µL; any value over 5 µL seems to be sufficient.
Save Now	This button applies the indicated values and saves the current settings to the Sun computer.
Additional Parameters	This button expands the SAMPLE Def. pane and provides the following parameters:
SampleWellRate	The rate, in mL/min, at which samples are aspirated from, and dispensed to, the sample container. A typical default is 4.
SampleHeight	The height (in units of 0.1 mm) above the rack at which the needle is placed before aspirating the sample. A value of zero should place the needle against the metal rack; a value of 25 (2.5 mm) is commonly used (but only if Sample Depth is set to NOSEEK. Because this may coat the outside of the needle with sample, the needle rinse station should be recalibrated to wash both the inside and outside of the needle.
SampleDepth	The depth of the liquid sample in the sample container. This value is used to allow the needle to follow the sample amount down as sample is withdrawn from the container. This parameter is commonly set to NOSEEK (especially when the Sample Height is set to 25) so the needle does not move down as the sample is aspirated.
NeedleRinseVolume	The volume of rinse solvent used to wash the needle. The default (and maximum usable value) is to set this equal to the syringe volume. Values that are too small could lead to increased carryover from one sample to the next. Values that are too large increase rinse solvent usage and increase the turnaround time.
NeedleRinseRate	The rate at which the syringe aspirates and dispenses rinse solvent through the needle. The default (and maximum usable rate) is 16 (mL/min).
Mix Volume	Defined but not yet implemented.
Mix Time	Defined but not yet implemented.
Mix Flow Rate	Defined but not yet implemented.
Mix Height	Defined but not yet implemented.

Rack Def. Pane

Use the Rack Def. pane (shown in Figure 105) to set up sample racks on the Gilson Liquid Handler.

The settings for racks 1 to 5 must reflect what racks exist on the system; otherwise, severe damage to the needle (probe) can result.

Main Control

Use the Main Control pane (shown in Figure 106) to control the Liquid Handler hardware.

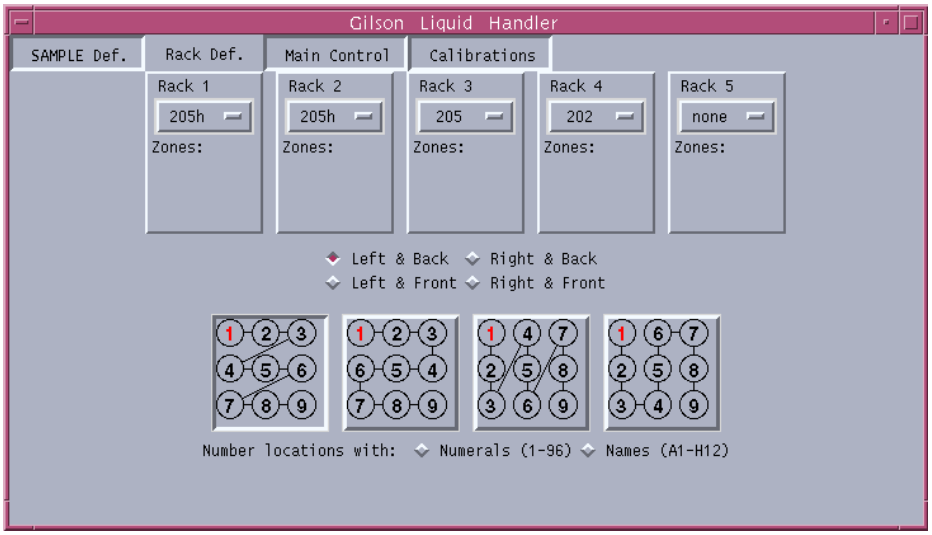


Figure 105. Rack Definition Window

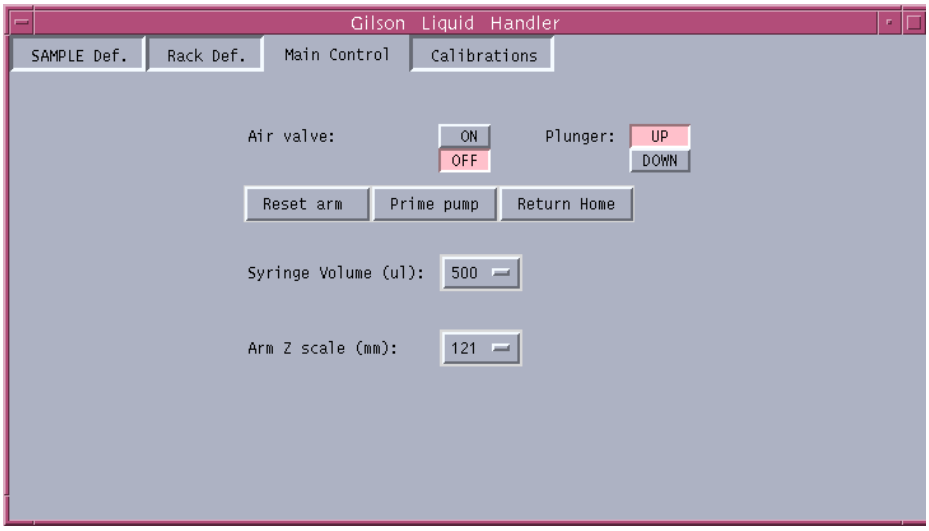


Figure 106. Main Control Window

This pane contains the following fields:

Air valve	Sets the Air valve to ON or OFF. The default is OFF, which allows the valve to be triggered by the VNMR automation software during normal operation. The valve provides gas pressure to the probe outlet port for pressure-assisted sample withdrawal. The gas pressure works in conjunction with gravity and the syringe to speed sample withdrawal and reduce cavitation. For normal operation, set Air valve to OFF. To provide continuous gas pressure to the probe (for example to empty the probe), set Air valve to ON.

Plunger	Moves the syringe plunger up or down. During normal operation, Plunger is set to UP, which is the default. When replacing the syringe, select DOWN; after the new syringe is in place, select UP.
Reset arm	Resets the Liquid Handler controller after an error or fault. If an error or fault occurs while the VAST autosampler is running, click on the Reset arm button to reset the Liquid Handler controller.
Prime pump	Moves the needle to the rinse station and runs the pump priming cycle, which takes about 3 minutes. Use this button to prime the pump after switching to a new solvent or after the system has been moved. When priming the pump, make sure no bubbles are in the inlet tubing to the barrel.
Return Home	Returns the arm to the predefined home position.
Syringe Volume	The syringe volume, in µL. This number must match the value printed on the syringe module.
Arm Z scale (mm)	Sets the arm Z scale value to accommodate the Liquid Handler probe (needle) module installed on the system. The scale from 0 to 220 mm is printed on the side of the vertical arm assembly. This value is normally set at installation; however, if you replace or adjust the position of the Liquid Handler vertical arm assembly, be sure to update the Arm Z scale value.

Calibrations

Use the Calibrations pane (shown in Figure 107) to calibrate the various positions of the Liquid Handler arm and probe (needle). The X, Y, and Z adjustments work in real time, so you must watch the probe move as you make adjustments.

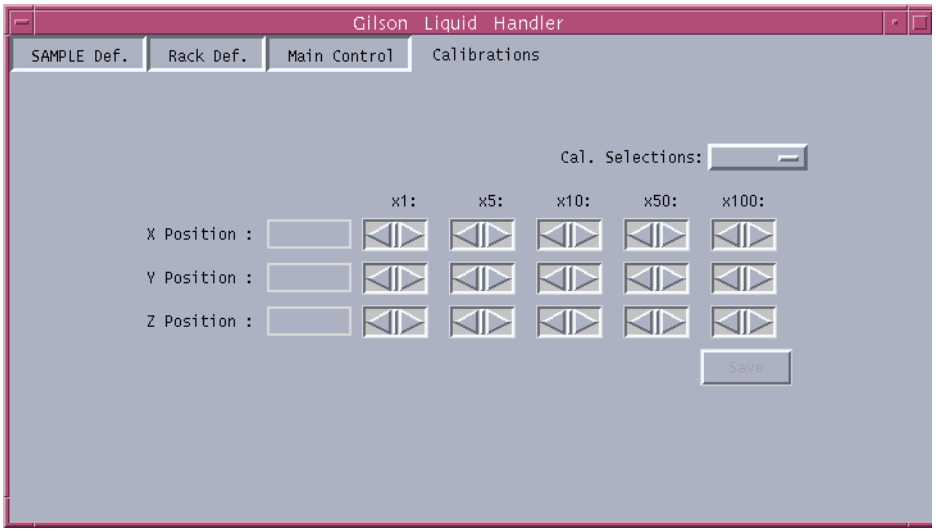


Figure 107. Calibrations Window

1. In the **Cal. Selections** menu, select rinse, sample, or inject.

2. Use the X and Y adjustment arrows to center the Liquid Handler probe over the position you are calibrating.
3. Use the Z adjustment arrows to calibrate the height of the Liquid Handler probe as follows:
   - *rinse* – For an *inside* wash, lower the needle until the tip is centered directly above the center of the rinse station. For an *inside and outside* wash, lower the needle until the tip is inserted several centimeters into the rinse station (depending on how much of the outside of the needle is contaminated; refer to the Sample Height parameter).
   - *rack* – Lower the needle below the level of the sample. Keep in mind that the needle lowers as it draws sample out of the container, unless the NOSEEK option is used for Sample Depth.
   - *inject* – Lower the needle 0.1 mm (x1) at a time until the needle makes snug contact with the injector port. If the needle begins to bow, back off until the needle is straight.
4. After a position is calibrated, click on **Save**. Then select another position, until all three positions are calibrated.

   The values of X, Y, and Z for the center of the injector manifold, and the values of X and Y for the racks, are written into the file `/vnmr/asm/racksetup`. The XYZ coordinates of the rinse station are stored only in the Liquid Handler memory.

   If bubbles appear in the transfer line (between the needle and the syringe) as the sample is withdrawn, this usually indicates that the needle is not calibrated to go deep enough into the inject port.

10.11 Customizing the enter Window for VAST

The file `entervast.conf`, located in `/vnmr/asm`, controls the appearance of the `enter` window, when `Sample Changer` is set to `VAST` in the VNMR configuration window.

The `entervast.conf` file uses information in the files `experiments.vast`, `solvents.vast`, `users.vast` and `protocol.vast` to determine which buttons are displayed. If you want to define protocols for injecting and removing samples, you will also need to uncomment the *infield* line in `entervast.conf`. The `experiments.vast` file typically points to the `autoscout` macro (among others).

After you click `Exit` and `Save` in the `enter` program, the text file to be used by `autogo` is written out. This text file (the `enter` queue) can be edited, if desired, before running `autogo`. For further information on customizing the `enter` program, refer to the manual *VNMR User Programming*.

An example of an `autoname` value that is especially useful for VAST is

```
autoname='sucrose_R%RACK:%_Z%ZONE:%_S%SAMPLE#:%_'
```

10.12 Files that Control VAST Operation

The files that control VAST automation are located in the directory `/vnmr/asm`.

`current`	contains information that indicates the current sample in the magnet. Additionally, the `sampleInfo` file (a link to `samp0` to `samp9` files) is appended into this file.
`default`	contains all the possible parameters, set to appropriate default values.
`info`	directory that contains the sample definition files.
`racks`	contains the supported Gilson rack definition files (e.g., code_205.grk). This directory also contains the file (`rackInfo`) used by the `gilson` program that defines the graphical characteristics of the racks.
`racksetup`	contains the injector and all racks and alignment values.
`samp0` to `samp9`	are in the info directory and contain the parameters and values as set by the user (using the gilson program) to override any defaults. The `gilson` program modifies the parameter values in these files. These parameters define the conditions for injecting and removing a sample from the probe.
`tcl`	contains the standard set of Tcl scripts provided by Varian NMR.
`protocols`	a directory to hold VAST protocols.

10.13 Writing VAST Protocols

This section describes the elements for programming custom VAST protocols. Some familiarity with the Tcl programming language will be useful. Sample protocols are in the `/vnmr/asm/protocols` directory.

gWriteDisplay **Display message on Gilson display (8 characters maximum)**

Syntax: `WriteDisplay message`

Arguments: `message` is a string of 8 characters maximum.

Description: Displays the string message on the LED display of Gilson 215.

Examples:
```
gWriteDisplay "Rinse"
set msg [format "Samp: %d" $SampleNumber]
gWriteDisplay "$msg"
```

gMoveZ2Top **Move needle to further most upward position**

Syntax: `MoveZ2Top`

Description: Moves the Gilson 215 needle to the fully retracted position.

Examples: `gMoveZ2Top`

gMove2RinseStation **Move to Rinse Station**

Syntax: `gMove2RinseStation`

Description: Moves the Gilson 215 needle to the Gilson's Rinse Station and lowers the needle. Note the Rinse Station position is maintained within the Gilson's internal EEPROM.

Examples: `gMove2RinseStation`

gFlush **Draws from the solvent reservoir and expels it through the needle**

Syntax: `gFlush Volume InFlow OutFlow`

Arguments: `Volume` is the volume in µl.

`Inflow` is the flow rate in ml/min in which solvent is drawn from solvent reservoir; if set to zero, then no solvent in drawn.

`OutFlow` is the flow rate in ml/min in which the solvent is expelled through the Gilson needle. If Zero, then no solvent is expelled.

Description: Draws the given volume from the solvent reservoir at the given flowrate and expels the same volume of solvent through the Gilson needle at given flowrate.

Examples:
```
gFlush gFlush $NeedleRinseVolume $NeedleRinseRate $NeedleRinseRate
gFlush $RinseVol $MaxFlow $ProbeFastRate
```

gMove2InjectorPort **Move to Injector port**

Syntax: `gMove2InjectorPort`

Description: Moves the Gilson 215 needle to the Gilson's Injector Port and lowers the needle.

Examples: `gMove2InjectorPort`

gDelayMsec **Delays TCL instruction execution**

Syntax: `gDelayMsec delaytime`

Arguments: `delaytime` is the time in milliseconds to delay Tcl execution.

Description: Delays Tcl instruction execution for the given number of milliseconds.

Examples: `gDelayMsec 500`

`gDelayMsec 1000` (sets a 1 second delay)

gSetContacts **Sets output contacts on Gilson 215**

Syntax: `gSetContacts contact# state`

Arguments: `contact#` is the contact number (`1` to `4`) to connected or disconnected.

`state` is `1` for connected or `0` for disconnected.

Description: Sets the Gilson's output contacts to connected or disconnected.

Examples: `gSetContacts 2 1` switches the gas valve to put pressure on NMR Probe.

`gSetContacts 2 0` switches the gas valve off.

gAspirate **Draw in liquid through Gilson needle**

Syntax: `gAspirate volume flow Zspeed`

Arguments: `volume` is the volume in µl to draw.

`flow` is the flowrate in ml/min.

`Zspeed` is the speed in mm/sec to lower Gilson needle while drawing in liquid through the needle. If `Zspeed` is set to zero, the needle is not lowered.

Description: Draws the specified volume in through Gilson needle at the given flowrate, with needle lowering at the speed given. The speed depends on the flowrate and sample well dimensions.

Examples: `gAspirate 250 4 0.0`
`gAspirate $SampleVolume $SampleWellRate $SampleZSpeed`

gDispense **Expel liquids though Gilson needle**

Syntax: `gDispense volume flow Zspeed`

Arguments: `volume` is the volume in µl to draw.

`flow` is the flowrate ml/min.

`Zspeed` is the speed in mm/sec to lower Gilson needle while expelling liquid through the needle. If Zspeed is zero, the needle is not lowered.

Description: Expels the specified volume out through Gilson needle at the given flowrate, with needle lowering at the speed given. Speed depends on flowrate and sample well dimensions.

Examples: `gDispense 250 4 0.0`
`gDispense $SampleVolume $ProbeFastRate 0.0`

gMoveZ **Move Gilson needle to a Z position**

Syntax: `gMoveZ Z_location`

Arguments: `Z_location` is the Z location in tenths of a millimeter.

Description: Move Gilson needle to the given Z location.

Examples: `gMoveZ $SampleZTop`

gMove2Sample **Move Gilson needle to sample well location**

Syntax: `gMove2Sample  Rack Zone Sample`

Arguments: `Rack` is the rack location 1 to 7.

`Zone` is the rack zone, which is rack dependent.

`Sample` is the sample tray number.

Description: Move Gilson needle to the sample well location.

Examples: `gMove2Sample $RackLoc $SampleZone $SampleNumber`

gInjector2Load **Switch Rheodyne injector valve to the load position**

Syntax: `gInjector2Load`

Description: Switches the Rheodyne injector valve to the load position.

Examples: `gInjector2Load`

gInjector2Inject Switch Rheodyne injector valve to the Inject position

Syntax: `gInjector2Inject`

Description: Switches the Rheodyne injector valve to the inject position.

Examples: `gInjector2Inject`

gCurrentSyrVol **Return current liquid volume in syringe**

Syntax: `SetCurrentVolume [gCurrentSyrVol]`

Arguments: `CurrentVolume` is the current volume in µl in Gilson syringe pump.

Description: Returns the current volume in µl in Gilson syringe pump.

Examples:
```
SetCurrentVolume [gCurrentSyrVol]
```

gAspirateAsync Draw in liquid through Gilson needle

Syntax:
```
gAspirateAsync volume flow Zspeed
```

Arguments: `volume` is the volume in µl to draw.

`flow` is the flowrate in ml/min.

`Zspeed` is the speed in mm/sec to lower Gilson needle while drawing in liquid through the needle. If Zspeed is zero the needle is not lowered.

Description: Draws the specified volume in through Gilson needle at the given flowrate, with needle lowering at the speed given. This function returns immediately, so other operation can be accomplished while aspirating. The speed depends on flowrate and sample well dimensions. Use `gStopTestAll` before performing any axis moves.

Examples:
```
gAspirateAsync 250 4 0.0
```

`gAspirateAsync $SampleVolume $SampleWellRate $SampleZSpeed` (starts aspiration operation)

`gSetContacts relay state` (while still aspirating switch a relay)

`gStopTestAll` (now wait for syringe operation to complete before moving)

`gilMoveZ2Top` (move needle up)

`gMove2InjectorPort` (move to injector port)

gDispenseAsync Expel liquids though Gilson needle

Syntax:
```
gDispenseAsync volume flow Zspeed
```

Arguments: `volume` is the volume in µl to draw.

`flow` is the flowrate in ml/min.

`Zspeed` is the speed in mm/sec to lower Gilson needle while expelling out liquid through the needle. If `Zspeed` is zero the needle is not raised.

Description: Expels the specified volume out through Gilson needle at the given flowrate, with needle lowering at the given speed. This function returns immediately, so other operation can be accomplished while dispensing. Speed will depend on flowrate and sample well dimensions

Use `gStopTestAll` before performing any axis moves.

Examples:
```
gDispenseAsync 250 4 0.0
```

`gDispenseAsync $SampleVolume $SampleWellRate $SampleZSpeed` (start dispensing operation)

`gSetContacts relay state` (while still aspirating switch a relay)

`gStopTestAll` (now wait for syringe operation to complete before moving)

`gilMoveZ2Top` (move needle up)

`gMove2RinseStation` (move to rinse station)

gStopTestAll **Wait for all axis motion and syringe operation to complete**

Syntax: `gStopTestAll`

Description: Return when all axes and syringe operations have completed.

Examples: `gStopTestAll`

`gAspirateAsync $SampleVolume $SampleWellRate $SampleZSpeed` (start aspiration operation)

`gSetContacts relay state` (while still aspirating switch a relay)

`gStopTestAll` (now wait for syringe operation to complete before moving)

`gilMoveZ2Top` (move needle up)

`gMove2InjectorPort` (move to injector port)

gZSpeed **Returns the Z speed (mm/sec) to follow the liquid while aspirating**

Syntax: `set zspeed [gZSpeed rackloc zone sample flowrate]`

Arguments: `zspeed` is the variable that contains the `zspeed`.

`rackloc` is the location of rack on the Gilson bed.

`zone` is the sample zone.

`sample` is the sample number.

`flowrate` is the flowrate for sample (ml/min).

Description: Return when all axes and syringe operations have completed.

Examples: `set SampleZSpeed [gZSpeed $RackLoc $SampleZone $SampleNumber $SampleWellRate]`

gMix **Mix a Sample in place**

Syntax: `gMix rackloc zone sample height mixvolume mixtimes mixflow`

Arguments: `rackloc` is the location of rack on the Gilson bed.
`zone` is the sample zone.
`sample` is the sample number.
`height` is the height above bottom of tube to place needle during mixing operation.
`mixvolume` is the volume that is aspirated and dispense for mixing.
`mixtimes` is the number of time the aspirating and dispensing are done.
`mixflow` is the flowrate for mixing sample (ml/min).

Description: Mixes a sample in the given sample position by aspirating the sample into the syringe then dispensing back into the sample well. This is done for the volume, flow, and number of times given.

Examples: `gMix $RackLoc $SampleZone $SampleNumber $SampleHeight $MixVolume $MixTimes $MixFlow`

gTransfer **Transfer sample from one well to another**

Syntax: `gTransfer racksrc zonesrc samplesrc heightsrc flowsrc rackdst zonedst sampledst heightdst flowdst`

Arguments: `racksrc` is the sample source location of rack.
`zonesrc` is the sample source zone.
`samplesrc` is the sample source number.

`heightsrc` is the source height above bottom of tube to place needle.
`flowsrc` is the source flowrate for sample (ml/min.)
`rackdst` is the sample destination location of rack.
`zonedst` is the sample destination zone.
`sampledst` is the sample destination number.
`heightdst` is the destination height above bottom of tube to place needle.
`flowdst` is the destination flowrate for sample (ml/min).

Description: Transfer the source sample to the destination sample location.

Examples:
```
gTransfer $XferLoc $XferZone $ZferNumber $XferHeight
$XferFlowRate $RackLoc $SampleZone $SampleNumber
$SampleHeight $SampleWellRate
```

gTubeX — Returns the X location of the sample tube

gTubeY — Returns the Y location of the sample tube

gMoveZLQ — Move until liquid is detected

gMove2LiqLevel — Move needle to the liquids level

Syntax:
```
gMove2LiqLevel rackloc zone sample height depth
```

Arguments:
`rackloc` is the location of rack on the Gilson bed.
`zone` is the sample zone.
`sample` is the sample number.
`height` is the height above the tube bottom to initially place the needle.
`depth` is the depth that the needle is extended into the sample. If `depth` is equal to NOSEEK then no further action is taken. If `depth` is not equal to NOSEEK then the needle is moved down from the detected liquid level or the height to the depth. The needle for safety reason will not be placed below the tube bottom or above the tube top as specified the Gilson rack definition file.

Description: Moves needle down into sample based on height and depth.

Examples:
```
gMove2LiqLevel $RackLoc $SampleZone $SampleNumber
$SampleHeight $SampleDepth
```

```
gMove2LiqLevel 1 1 10 30 NOSEEK
```

This example places needle 3 mm from the bottom of the sample tube.

```
gMove2LiqLevel 1 1 10 180 100
```

This example moves needle down attempting to detect liquid level using the Gilson liquid sensor until the liquid is detected or needle is 18-mm above the sample tube bottom. Then the needle is extended down another 10-mm (depth) into the sample.

gRackZoneSequenceOrder — Sample sequencing order by zone

Syntax:
```
gRackZoneSequenceOrder rackloc zone startloc pattern
```

Arguments:
`rackloc` is the location of rack on the Gilson bed.
`zone` is the sample zone.
`startloc` is the position of sample number 1 (NW, NE, SW, SE).
`pattern` is the sequencing pattern (HST, HZZ, VST, VZZ).
`HST` is the horizontal straight.

HZZ is the horizontal zig-zag.
VST is the vertical straight.
VZZ is the vertical zig-zag.

Description: Defines the sample sequencing order for the rack and zone.

Examples:
```
gRackZoneSequenceOrder $RackLoc $SampleZone NW HST
```

gRackSequenceOrder Sample sequencing order by rack

Syntax:
```
gRackSequenceOrder rackloc startloc pattern
```

Arguments: `rackloc` is the location of rack on the Gilson bed.
`startloc` is the Position of Sample number 1 (NW, NE, SW, SE).
`pattern` is the Sequencing pattern (HST, HZZ, VST, VZZ).
HST is the horizontal straight.
HZZ is the horizontal zig-zag.
VST is the vertical straight.
VZZ is the vertical zig-zag.

Description: Defines the sample sequencing order for all zones on the rack.

Examples:
```
gRackSequenceOrder $RackLoc NW HST
```

gRackLocTypeMap Maps the type of rack to a location on the Gilson 215 bed

Syntax:
```
gRackLocTypeMap rackloc racktype
```

Arguments: `rackloc` is the location of rack on the Gilson bed.
`racktype` is the Gilson rack type (205, 205H, 505, etc).

Description: Maps the type of rack to a location on the Gilson 215 bed. These calls are created by the Gilson program and place in the `/vnmr/asm/info/racks` file.

Examples:
```
gRackLocTypeMap 1 205
```

This example shows that in position one there is a 205 rack.

ResumeAcq Allows acquisition to start while Tcl script still runs

Syntax:
```
ResumeAcq
```

Description: Sends a signal to console that sample change is complete, this allows acquisition to continue even while the Gilson is still performing other operations.

Any operation must be complete by the time the next sample is to be changed. Otherwise, unpredictable results will occur.

Examples:
```
ResumeAcq
```

gPuts Diagnostic output for Tcl scripts

Syntax:
```
gPuts "'string' $parmeter \n"
```

Description: Prints function for diagnostic output. Output is inhibited when Tcl parameter `debug` is zero (default). Output is enabled when Tcl parameter `debug` is 1

Examples:
```
gPuts "My parmeter value is $myparmeter \n"
```

Outputs `My parameter value is 42` and sets debug 1.

Chapter 11. PFG Modules Operation

Sections in this chapter:

- 11.1 “Configuring the Software,” this page
- 11.2 “PFG Amplifier Operation,” on page 372
- 11.3 “Shimming PFG Systems,” on page 374
- 11.4 “Setting Up Software for Imaging Pulse Sequences,” on page 374
- 11.5 “Homospoil Gradient Type,” on page 375
- 11.6 “Gradient Shimming,” on page 376

This chapter covers operation of the Varian Performa pulsed field gradient (PFG) modules. The Performa I, Performa II, Performa III, and Performa XYZ PFG modules add new capabilities to high-resolution liquids experiments on most systems (only the Performa I is available on the *GEMINI 2000*). Single-axis systems apply a gradient in B_0 at programmed parts of the pulse sequence. This gradient can perform several functions, including solvent suppression and coherence pathway selection. Triple-axis systems can also perform solvent suppression and coherence pathway select as well as imaging.

The gradient system produces pulses with strengths of up to 65 gauss/cm. The gradient subsystem produces an intense gradient for a time and then returns to spectroscopic conditions quickly. The relevant parameters are the gradient strength and stability, the duty cycle of the gradient system, and the time to recover to spectroscopic conditions.

The PFG module installation manual covers installation, calibration, and test procedures for PFG modules.

11.1 Configuring the Software

When the PFG module is installed, values are set in the CONFIG window for PFG using the label X Axis, Y Axis, Z Axis (for *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, PFG is selected in the CONFIG window by setting the choice to `'Present'`, which selects “Performa I”). Each axis has a number of choices including Performa I, Performa II/III, Performa III, and Performa XYZ. The CONFIG window is opened by entering the `config` command. The configuration process is described in the manual *VNMR and Solaris Software Installation*.

From the choices made in the CONFIG window, the parameter `gradtype` is set as a string of three characters (e.g., `gradtype='nnp'`):

- The first character is the gradient for the X axis, the second for the Y axis, and the third for the Z axis.
- Each axis is `'n'` (None choice in CONFIG window), `'w'` (WFG+GCU), `'l'` (Performa I), `'p'` (Performa II/III), `'q'` (Performa II/III + WFG), `'t'` (Performa XYZ), `'u'` (Performa XYZ + WFG), `'s'` (SIS (12 bit), or `'h'` (Homospoil). WFG

stands for the waveform generation, and GCU stands for the gradient compensation unit. Homospoil is functional only on the Z axis.

Standard PFG operation with the single-axis probe uses the following parameter settings:

- `gradtype='nnl'` is for Performa I hardware with only the Z channel available.
- `gradtype='nnp'` is for Performa II hardware with only the Z channel available.

Performa XYZ operation with the triple-axis probe uses the following parameter settings:

- `gradtype='ttt'` is for Performa XYZ hardware with all three channels available.
- `gradtype='nnt'` is for Performa XYZ hardware with only the Z channel available.

After the software is configured, the system is ready to use. Table 51 summarizes the commands and parameters associated with PFG.

Table 51. Pulsed Field Gradients Commands and Parameters

Command	
`createtable`	Generate system gradient table
`grecovery`	Eddy current testing
`setgcoil<(file)>`	Assign sysgcoil configuration parameter
`updtgcoil`	Update gradient coil
Parameters	
`boresize` {number, in cm}	Magnet bore size
`gcal` {number, in G/cm-DAC}	Gradient calibration constant
`gcoil` {string}	Current gradient coil
`gmax` {number, in gauss/cm}	Maximum gradient strength
`gradtype*`	Gradients for x, y, and z axes
`gxmax,gymax,gzmax*`	Maximum gradient strength for each axis
`pfgon*`	PFG amplifiers on/off control
`sysgcoil` {string}	System gradient coil
`trise` {number, in sec}	Gradient rise time

* `gradtype` (3-character string from 'n','w','l','p', 'q', 's','t','u','h')
`gxmax,gymax,gzmax` {number, in gauss/cm)
`pfgon` (3-character string from 'n' and 'y')

11.2 PFG Amplifier Operation

The PFG current amplifier is left on during the experiment, eliminating the need for current blanking. Although the PFG amplifiers are quiet, they do produce a small amount of quiescent current, resulting in a shift of the Z1 shimming. The Performa XYZ PFG amplifier produces a small amount of quiescent current into each of the X, Y, and Z gradient coils, resulting in a change to the X1, Y1, and Z1 shimming.

Although leaving the amplifier on to have the shimming stable is a reasonable procedure, it may be disabled by the global string parameter `pfgon` to check for noise sources or to change probes. `pfgon` is a three-character string with the first character controlling the X channel, the second controlling the Y channel, and the third controlling the Z channel. The value for each channel is `y` or `n` (e.g., `pfgon='nny'` turns on the PFG amplifier on the Z channel only). A `su` or `go` command must be sent to activate `pfgon`.

It is useful to translate the gradient control in DAC units to G/cm by a constant that represents G/cm-DAC units. The parameter `gcal`, a user global real-valued parameter, makes this translation.

The amplifier system is well-behaved during power up and other exceptional conditions. For example, if the console is on and the current amplifier is subsequently switched on, the unit warms up but its output is disabled by the internal logic. During the setup, the interface transmits a zero to the unit. The output is enabled whenever a `su` or `go` operation is requested with `pfgon='nny'`. The enable logic in the current driver is edge-triggered so that the output current is not enabled unless an explicit command is sent.

If the amplifier was on and the console power cycled or rebooted, the AP bus control of the enable is off, disabling the current. An `su` then restores normal functioning. If an experiment requiring the gradient is started without the `su` command, the experiment fails.

As an extra safety precaution with Performa II systems, pressing the reset button on the interface board zeroes the main DAC. Similarly, a reset aborts zero but does not disable the output current. Using `pfgon='nnn'`, or setting the amplifier standby switch disables amplifier output.

PFG amplifiers have a series of lights to enable you to diagnose operation:

- Performa I amplifier lights:

POWER	Power is on.
ON	Amplifier is active.
ACK	AP response.
DATA	Flickers when data is sent to it, indicating the interface to the amplifier from the system is working.
ERR	Duty cycle exceeded or internal overload.
LOAD	Amplifier sees a short or open circuit. Check probe and probe connections.

- Performa II and Performa III amplifier lights:

POWER	Power is on.
ON	Amplifier is active.
DATA	Flickers when data is sent to it, indicating the interface to the amplifier from the system is working.
HITEMP	fault: internal thermal overload.
ERROR	Duty cycle exceeded.
WARMUP	Warming up (operation not recommended).
LOAD	Amplifier sees a short or open circuit. Check probe and probe connections.

- Performa XYZ amplifier lights:

POWER	Power is on.
ON	Amplifier is enabled.
DATA	Flickers when data is sent to it, indicating the interface to the amplifier from the system is working.
PULSE	Gradient pulse is active.
ERROR	Duty cycle exceeded, open load, or blown coil fuse.

11.3 Shimming PFG Systems

The procedures in this apply to the Performa I, Performa II, and the Performa XYZ systems. Once in operation, leave the amplifier on while using the gradient system, to allow the amplifier to reach a long-term equilibrium.

Performa I and Performa II

1. Enter **`pfgon='nnn' su`** to turn off the amplifier. Verify a drop in the lock level from the small dc zero current from the amplifier.
2. Shim the system to the desired level.
3. Enter **`pfgon='nny' su`** to turn on the amplifier. The shimming changes from the small dc offset current.
4. Adjust Z1 to restore the homogeneity. The lock level should have identical stability on the meter.

This two-stage approach is not strictly necessary, but it does separate any problems that might arise.

Performa XYZ

1. Prepare the amplifier by moving the switch from STANDBY position to ON.
2. Enable the amplifier by entering **`pfgon='yyy' gradtype='ttt' su`** (or **`gradtype='uuu'`** for Performa XYZ with waveshaping). The yellow RUN lights turns on. Shim the system to the desired level.

11.4 Setting Up Software for Imaging Pulse Sequences

After the gradient configuration has been selected, to make use of the imaging, obliquing, and variable angle pulse sequences and pulse sequence statements, calibrate the gradients, create a gradient table, and set the configuration parameter `sysgcoil` to the desired gradient coil. Each of these steps is explained in the next sections.

Calibrating the Gradients

Follow the steps in the “PFG Test Procedure” section in the PFG installation manual to calibrate the gradients. Calibrate each axis and save the values for the maximum gradient strength in gauss/cm. These values will be needed when creating a gradient table.

Creating a Gradient Table

A gradient table should be created for each gradient coil/amplifier combination. Run the `creategtable` macro, then enter the following information:

- Maximum gradient strength of each axis
- Gradient rise time
- Gradient bore size

Setting the System Gradient Coil

Once a gradient table has been created, it can then be configured in the system. Any number of gradient tables can be created, although the `config` program can only handle 30. Configuring a table in the system means setting the system gradient coil parameter, `sysgcoil` to the name of the gradient table. To set `sysgcoil`, use the CONFIG window or the `setgcoil` macro.

- In the CONFIG window, select the Gradient window to initially configure the gradients. Notice the field for Imaging Gradient Coil. Select the desired table from the list of choices. If the name does not exist, it means a table has not been created. For a list of configuration items, see the manual *VNMR and Solaris Software Installation*.
- To configure a gradient table using `setgcoil`, enter `setgcoil(file)`, where `file` is the file name of the table (e.g. `setgcoil('tbl')`). This updates the values of `sysgcoil` and `gcoil` as well as related parameters in the current experiment.

After a system gradient coil has been configured, any experiments that have a `gcoil` parameter will automatically be updated whenever that experiment is "joined" or new parameters are retrieved. When the `gcoil` parameter is updated, all the parameters corresponding to the entries in the gradient table file (`gxmax`, `gymax`, `gzmax`, `trise`, and `boresize`) are also updated. These parameters are then used by the pulse sequence. `gxmax`, `gymax`, and `gzmax`, in particular, are used by the obliquing PSG elements to convert gauss/cm to DAC units. To create and set a `gcoil` parameter for an experiment parameter set, type `updtgcoil`.

11.5 Homospoil Gradient Type

Starting with VNMR 6.1A, it is possible to use homospoil (room temperature Z1 shim coil) as a general gradient type. It does not require the use of a pulsed field gradient module and thus is available on systems without PFG. Homospoil gradients are implemented only on the Z axis.

When homospoil is switched on in a pulse sequence, the shim current is set to maximum for a given period of time. Homospoil control within a pulse sequence is done in the following manner:

- To use homospoil as a quick homogeneity spoil, use `hsdelay`. This is the traditional homospoil method, and is usually done at the beginning of a relaxation recovery delay (e.g., `hsdelay(d1)`). The parameter `gradtype` is ignored. See the *User Programming manual* for details of how to use `hsdelay`.
- To use homospoil as a general gradient type, first select the homospoil gradient type. Enter **`config`** and under **Gradients** select **Homospoil** (this sets `gradtype='nnh'`). The parameter `pfgon` is ignored, since a separate gradient amplifier is not needed. Homospoil is then triggered by gradient statements such as `rgradient('z',gzlvl1)`. If the value of `gzlvl1` is non-zero, homospoil is switched on; if the value of `gzlvl1` is zero, homospoil is switched off. Only one sign and strength of gradient current is available during a pulse sequence, and is set by hardware.

Homospoil gradients may be switched on only for a limited period of time, usually 20 ms. This time limit is determined by hardware in UNITY*INOVA* and UNITY*plus* systems (see Table 52 for system configurations). Check your pulse sequences to ensure this time limit is not exceeded.

Table 52. Homospoil Control

System	*Shim Supply*	*Homospoil Time Limit*
UNITY*INOVA*	Varian 14	20 ms/200 ms[a]
UNITY*INOVA*	Varian 18 to 40	20 ms/200 ms[a,b]
UNITY*INOVA*	RRI Ultrashims	20 ms/200 ms[a]
UNITY*plus*	Varian 13	20 ms/200 ms[c]
UNITY*plus*	Varian 18 to 40	20 ms/200ms[a,b]
UNITY*plus*	RRI Ultrashims	20 ms/200 ms[a]
UNITY/VXR-S	Oxford 18	Weak homospoil[d]
MERCURY-VX, *MERCURY*	Varian 14	No time limit[e]
GEMINI 2000	Varian 13	No homospoil

a. Hardware upgrade to 200 ms with the Automated Deuterium Gradient Shimming module is required for compatibility with ^{2}H gradient autoshimming.
b. Hardware adjustment required for both ^{1}H and ^{2}H gradient autoshimming. Adjust homospoil potentiometer resistor labeled HOMO (blue square) on front of Z0/Z1 board to maximum in either direction for maximum homospoil gradient strength.
c. Hardware upgrade required for both ^{1}H and ^{2}H gradient autoshimming. Homospoil gradient strength is too weak for autoshimming without hardware upgrade.
d. Hardware upgrade not available. Homospoil gradient strength is too weak for gradient autoshimming.
e. A homospoil time limit of 20 ms is set by software for `hsdelay`.

The behavior of homospoil gradients is quite different from that of a pulsed field gradient. The gradient strength is much weaker than the traditional PFG, and the recovery time is much longer because of eddy currents. The strength and recovery of the gradient depends on the shim coils and system hardware. Typically, these gradients are suitable only for profile-type experiments and unsuitable for gradient coherence-selection experiments such as GCOSY and GNOESY. For all gradient experiments, pulsed field gradients are preferred if available.

Homospoil gradients are suitable for ^{1}H and ^{2}H gradient shimming on some systems (see Table 52 for system configurations). The Automated Deuterium Gradient Shimming module (see *Getting Started* and *Accessories Installation* manuals) is required on UNITY*INOVA* and UNITY*plus* systems to upgrade the homospoil hardware for compatibility with deuterium gradient shimming.

11.6 Gradient Shimming

Gradient shimming provides rapid, automatic shim adjustment and applies to all systems with PFG or homospoil and gradient shimming software installed.

Proton gradient autoshimming with PFG is available on all systems configured with a PFG accessory. Deuterium gradient shimming is only available on UNITY*INOVA*, UNITY*plus*, *MERCURY-VX*, and *MERCURY* systems and not on *GEMINI 2000*, UNITY, and VXR-S systems because lock sample and hold capability is required to perform deuterium gradient

shimming. A hardware upgrade is also required to perform deuterium gradient shimming. System configuration requirements are summarized in Table 53.

Table 53. Gradient Shim Availability

System	*Gradient Shim Availability*
UNITY*INOVA*, UNITY*plus*	^{1}H or ^{2}H with PFG or Homospoil[a]
MERCURY-VX, *MERCURY*	^{1}H or ^{2}H with PFG or Homospoil[b]
UNITY, VXR-S, *GEMINI 2000*	^{1}H with PFG only

a Automated deuterium gradient shimming module required for deuterium gradient shimming with PFG or homospoil.

b Automated liquids/solids spinner controller and automated deuterium gradient shimming module required for deuterium gradient shimming.

Configuring Gradients and Hardware Control

1. Confirm that PFG or homospoil gradients are installed on your system. See the previous sections in this chapter.
2. Confirm that the gradients are active by checking that `gradtype` and `pfgon` are set appropriately for your system. Use `config` to change settings if necessary.
3. If you have the Ultra•nmr shim system, enable control of the shims from the Acquisition window, as described in the section “Shimming Using the Ultra•nmr Shim System” in the manual *Getting Started*.

Gradient Shimming Method

The full gradient shimming method consists of these steps:

1. Map the shims.
2. Perform autoshimming.

The shims must be mapped before autoshimming is used. Mapping the shims is necessary when a new probe is installed, but can be repeated at any time.

Table 54 summarizes gradient shimming commands and parameters.

CAUTION: **Spinning the sample during gradient shimming can cause motion artifacts.**

Mapping the Shims

Mapping the shims is necessary after installing a new probe. 90% H_2O is recommended for first time shimming on ^{1}H and 1%H_2O/99%D_2O is recommended for ^{2}H.

1. Insert a sample and find lock.
2. Stop sample spinning (**`spin=0`**). Disable sample changer control (**`loc='n'`**).
3. Adjust lock power, lock gain, and lock phase. Make coarse shim adjustments on Z1, Z2, X1, and Y1.
4. Use **`s2pul`** to find the 90° pulse for `tn='H1'` or `tn='lk'`, as appropriate.

Table 54. Gradient Shimming Commands and Parameters

Commands	
`dg2`	Display group of parameters.
`gmapshim<('files'|'quit')>`	Run gradient autoshimming, get files and parameters, quit.
`gmapsys*`	Enter Gradient Shimming System menu, make shimmap.
`gmapz<(mapname)>`	Get parameters/files for `gmapz` pulse sequence.
`* gmapsys<'shimmap'<,'auto'|'manual'|'overwrite'|mapname>`	
Parameters	
`d2`	Incremented delay for 1st indirectly detected dimension.
`d3`	Incremented delay for 2nd indirectly detected dimension; arrayed to two values
`gradtype*`	Gradients for x, y, and z axes
`gzlvl {DAC value}`	Pulsed field gradient strength
`gzsize {integer,1 to 8}`	Number of z-axis shims used by gradient shimming
`gzwin {0 to 100}`	Percentage of spectral window used by gradient shimming
`p1`	First pulse width—If > 0, it is used between the gradient pulses as a 180 refocusing pulse, and the gradients have the same sign.
`pfgon{'nny'` if on}	PFG amplifiers on/off control
`pw`	Pulse width; it can be <90° if p1=0.
`*` `gradtype` {3-character string from `'n','w','l','p','q','s','t','u','h'}`	

5. Enter **`gmapsys`** to display the Gradient Shimming System menu.

   Standard parameters are retrieved from `gmapz.par` the first time `gmapsys` is entered, or if a shimmap was previously made, parameters are retrieved from the current shimmap. If desired, enter **`gmapz`** to retrieve standard parameters from `gmapz.par`.

6. To set parameters for a particular gradient and nucleus, enter **`gmapsys`** and click on **Set Params** > **Gradient, Nucleus**, and then click on the appropriate button (**Pfg H1, Pfg H2, Homospoil H1,** or **Homospoil H2**). Next, set pw as follows:
   - For PFG, set **`pw`** to the 90° pulse or less.
   - For homospoil, set **`pw`** to 90° pulse and `p1` to 180° pulse.

7. Test the parameters. Enter **`gmapsys`** and click on **Set Params** > **Go, dssh**.

   You should see two profile spectra. If you don't, check that the gradients are active and check `pw`, `tpwr`, and `gain`.

8. To make a shimmap, again enter **`gmapsys`** and click on **Shim Maps** > **Automake Shimmap**. Enter a mapname (any string valid for a file name) at the prompt.

Starting Gradient Shimming

To start shimming as a system administrator, enter **`gmapsys`** and then click on **Autoshim on Z**. This button starts gradient shimming using current parameters, and displays the curve fit and shim adjustments for each iteration.

Quitting the Gradient Shimming System Menu

Enter **`gmapsys`** and click on **Quit** to exit from the gmapsys menu system. This also retrieves the previous parameter set and data, including any data processing done on the previous data set.

General User Gradient Shimming

For the general user, gradient shimming can be run from outside `gmapsys` from any experiment. Any one of the following methods is recommended for routine use:

- Click on **Main Menu** > **Setup** > **Shim** > **Gradient Autoshim on Z**. Parameters are retrieved from the current mapname, which is displayed at the start of shimming, and the spinner is automatically turned off. The curve fit and shim adjustments are not displayed. The previous parameter set and data are retrieved when shimming is finished. This button only functions after a shimmap is made.
- Enter **`gmapshim.`** This performs the same action as clicking on **Gradient Autoshim on Z**.
- Within automation parameter sets, use **`wshim='g'`** (UNITY*INOVA*, *MERCURY-VX*, UNITY*plus*, and *MERCURY* only).

To stop gradient shimming before it is completed, use one of the following methods:

- Click on **Main Menu** > **Setup** > **Shim** > **Quit Gradient Shim**. Quitting aborts the experiment and retrieves the previous parameter set and data.
- Enter **`gmapshim('quit')`**. This performs the same action as **Quit Gradient Shim**.
- Abort the acquisition with **`aa`** and click on **Cancel Cmd**. Then enter **`gmapshim('quit')`** to retrieve previous data set and parameters.

How Gradient Shimming Works

The basis of gradient shimming is differential phase accumulation from shim gradients during an arrayed delay. The phase is spatially encoded by a pulsed field gradient. Figure 108 shows the gradient shimming pulse sequence.

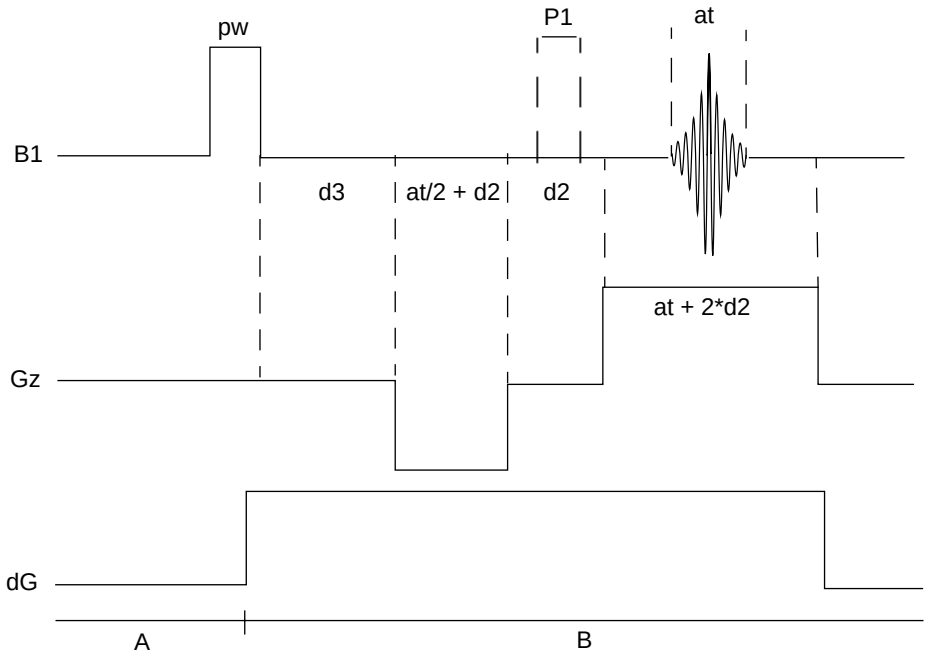


Figure 108. Gradient Shimming Pulse Sequence

The gradient shimming pulse sequence in Figure 108 is shown with `p1=0`, in which case `pw` can be set to a small flip angle. If `p1>0`, the pulse field gradients are both set to the same sign, and `p1` should be set to 180 ° and `pw` to 90°, so that rf inhomogeneities are refocused. `p1=0` is usually sufficient for most cases.

Phase accumulation from all gradients present is as follows:

ϕ = z Gz(-at/2 + t) + dG(d3 + at/2 + 3*d2 + t)

where `t` is the time during acquisition `at`, `Gz` is the z-axis pulsed field gradient strength, and `dG` is the sum of the shim gradient fields, shown as being on during relevant times in the pulse sequence.

The effect of the shim gradients `dG` can be isolated by arraying `d3` and taking the difference in the phases:

$\Delta\phi$ = ϕ2 - ϕ1 = dG*(d3[2] - d3[1])

For example, at a particular point, $\Delta\phi$ can be 2π * 100 Hz * 10 ms, or 2π radians. Thus, a pair of profiles with different `d3` values can be used to calculate the B_0 field along z.

The effect of any one shim gradient can be isolated by arraying the shim value, represented by `dG`, and taking the difference in the phase differences:

$\Delta(\Delta\phi)$ = $\Delta\phi$2 - $\Delta\phi$1 = dG2*(d3[2]-d3[1]) - dG1*(d3[2]-d3[1])
=(dG2 - dG1)*(d3[2] - d3[1])

Therefore, two pairs of profiles can be used to map out the effect of a shim. By arraying all the shim values, a set of phase difference maps or shim field maps can be constructed for a given shim set. Shimming can then be performed by constructing a background field map for the starting shim values ($\Delta\phi$) and fitting the result to the shim field maps. The calculations are quite fast, so the entire shimming process is usually limited by the data acquisition time, typically taking only a few minutes.

In practice, the phase is calculated from ϕ=arctan(x,y) from the real and imaginary values at each point in the spectrum, and $\Delta\phi$ is calculated from the difference in the phases of a pair of spectra with `d3` arrayed. Figure 109 shows an example of mapping the z1 shim.

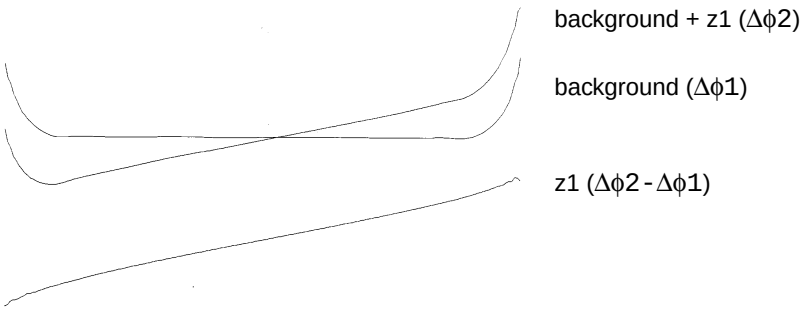


Figure 109. Mapping the z1 Shim

References

Van Zijl, P. C. M., et al. *J. Magn. Reson.* **1994**, *111* (Series A), 203–207.

Sukumar, S., et al. *J. Magn. Reson.* **1997**, *125* (Series A), 159–162.

Barjat, H., et al., *J. Magn. Reson.* **1997**, *125* (Series A), 197–201.

How Making a Shimmap Works

Automake Shimmap first runs an experiment that calibrates `gzwin` and `tof` to set the spectral window. Next, it runs an experiment with the shims arrayed to map the shims, and processes the experiment when done. Coarse shims are used if present. The parameters and data for the shimmap are stored in the file `userdir + '/gshimlib/shimmaps/' + mapname + '.fid'`. These parameters are retrieved the next time gradient shimming is run if the gradient shimming system menu is exited.

Displaying the Shimmap

After the shims are mapped, display the shimmap by entering **`gmapsys`** and clicking on **Display > Display Shimmap**. The shimmap display is a multicolored plot of the shimmap, with Z1 as #1 and Z2 as #2, and so on (see Figure 110).

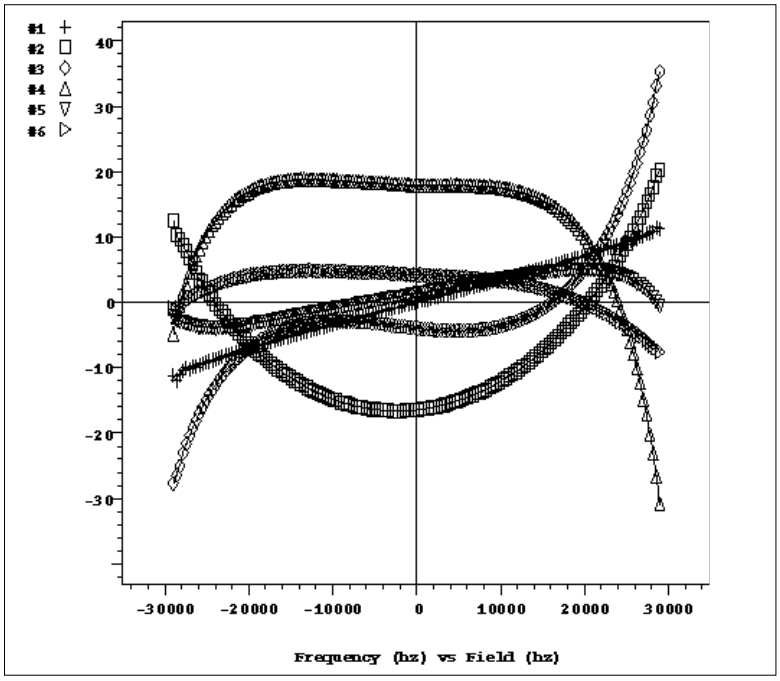


Figure 110. Shimmap Plot

The shimmap is specific to the probe used, and can also be dependent on sample volume for small volumes. The shimmap shows the actual field dependence of the shims, except for a dc offset added for display purposes. Good signal-to-noise in the shimmap is needed for the shimming to work well. Poor signal-to-noise might result in incorrectly set shims.

Calibrating gzwin (optional)

The parameter `gzwin` is the percentage of the spectral window used in calculating the field maps. `gzwin` should be adjusted only when making a new shimmap. If this parameter is not calibrated correctly, you may see excess noise data at the edge of the shimmaps, which corresponds to the region in the profile spectrum where the signal goes to zero. It is normal to have a few noise data points at the edge of the shimmap, but if it is more than a few data points (greater than 25% of the window), `gzwin` may be miscalibrated. This can occur if there is low signal-to-noise or if `gzwin` has not previously been calibrated for the current parameter set. If the gain is too high, “wings” will appear on the sides of the spectra and

may result in miscalibrated gzwin. This can also occur if there are multiple chemical shifts in the presence of a weak gradient.

Automatic Calibration of gzwin

Clicking the Automake Shimmap button causes the following actions:

1. Enter **gmapsys** and click on **Set Params** > **Find gzwin**, which calibrates gzwin and sets tof to center the window used for calculation.
2. Click on **Return** > **Shim Maps** > **Make Shimmap**, which makes the shimmap with the current values of gzwin and tof.

You may click through these steps separately to see if gzwin is calibrated correctly. The box cursors at the end of step 1 should be at either edge of the profile.

Manual Calibration of gzwin

Manual calibration of gzwin may be used to avoid noise spikes in the spectrum, or other artifacts. To manually calibrate gzwin, do the following:

1. Click on **Set Params** > **Go, dssh**. Wait until the experiment is done.
2. Enter **ds**, and set the box cursors near the edges of the profile.
3. Enter **gmapsys**, and click on **Set Params** > **Calculate gzwin**.
4. Click on **Return** > **Shim Maps** > **Make Shimmap**.

The parameter gzwin should be adjusted only when making a new shimmap. The calibrated value of gzwin is saved when the new shimmap is saved at the end of the mapping experiment. The same value of gzwin must be used in shimming as in making a shimmap, and should not be adjusted when shimming.

Shimmap Files and Parameters

The parameters and shimmap files saved under a mapname are retrieved when that mapname is retrieved. When reinserting a probe, reload the shimmap for that probe. If you are unsure if the shimmap is correct, make a new shimmap, which typically only takes a few minutes. The last parameters and files used are automatically retrieved the first time gmapsys is entered. If gmapsys is entered again, the parameters are not retrieved. Gradient shimming uses the current parameters after the pulse sequence is loaded (seqfil='gmapz').

Standard parameters can be loaded before making a shimmap by entering gmapz or by using the **Gradient,Nucleus** menu button. Parameters and files can also be explicitly loaded and distributed, as described in the following subsections:

Loading a Shimmap

To change shimmaps as a system administrator, do the following:

1. Enter **gmapsys**.
2. Click on **Shim Maps** > **Shimmap Files** > **Cd to Userdir**.
3. Select a file.
4. Click on **Load Shimmap** (loads the shimmap files gshim.list and gshim.bas from gshimlib/shimmaps/mapname.fid into gshimlib/data, but does

not load the parameters) or click on **Load Shimmap & Params** (loads shimmap files and parameters).

The general user can also change shimmaps by entering **`gmapshim('files')`** from any experiment to display the Gradient Autoshimming Files menu, and then clicking on either the **Load Shimmap** or **Load Shimmap &Params** buttons.

Distributing a Shimmap

The system administrator can copy a shimmap file from `vnmrsys/gshimlib/shimmaps` into the directory `/vnmr/gshimlib/shimmaps` so that the file is accessible to all users. To copy files, do the following steps:

1. Log in as **`vnmr1`**.
2. Enter **`gmapsys`**.
3. Click on **Shim Maps** > **Shimmap Files** > **cd to Userdir**.
4. Select a file.
5. Click on **Copy to Systemdir**.
6. Become the new user to be given access to gradient shimming.
7. Enter **`gmapsys`**.
8. Click on **Shim Maps** > **Shimmap Files** > **cd to Systemdir**.
9. Select a file.
10. Click on **Load Shimmap**.

How Automated Shimming Works

The shims must be mapped before gradient autoshimming is used. See “Mapping the Shims,” on page 377 for details.

When gradient shimming is run from the **`gmapsys`** menu, the curve fit plot is displayed for each iteration. The plot shows the raw data as #1 and the curve fit as #2 (see Figure 111).

Shim adjustments for each iteration are also displayed (see Figure 112) and have converged when the rms error number is less than 1.0. Gradient shimming continues until convergence or until a maximum of 10 iterations are reached.

If a shim goes out of range, the shim is set to maximum and shimming continues with the remaining shims. If convergence is then reached, shimming is tried once more with all Z shims and continues unless a shim goes out of range again.

If the parameter `gmap_z1z4` is set to `'y'`, then if `gzsize` is greater than 4, shimming is done first on Z1–Z4 and then proceeds with all shims specified by `gzsize`. Gradient shimming takes longer and goes through more iterations, but this may avoid the problem on some systems where a high-order shim (i.e. Z5, Z6) goes out of range because it contains impurities from lower-order shims. This parameter may be set at any time while shimming from `gmapsys`. In order to use this parameter in user autoshimming, set it before making a shimmap, or in the corresponding parameter set in `gshimlib/shimmaps`.

Deuterium Gradient Shimming

Deuterium gradient shimming is feasible for most deuterated solvents for which lock solvent has a single, strong deuterium resonance with sufficient signal.

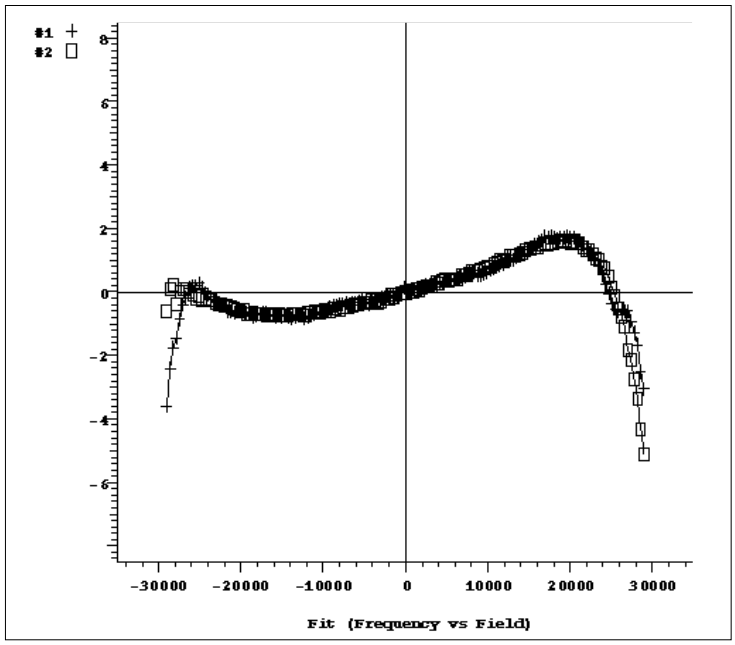


Figure 111. Curve Fit Plot

```
mapname 5mm_Triax_01
shimset 4  gzsize 6                          rms err 1.892

Shim    Offset     Old      New        Diff      Error
-------------------------------------------------------------
z1      800        -9405    -9269      -136      48
z2      800        -3118    -3104      -14       13
z3      3200       -4356    -4321      -35       37
z4      -3200      4049     4885       -836      104
z5      -3200      13443    14537      -1094     322
z6      3200       -15619   -12568     -3051     467
z7      3200       0        0          0         0
z8      3200       0        0          0         0
-------------------------------------------------------------
```

Figure 112. Display of Shim Adjustments for Each Iteration

The automated deuterium gradient shimming module is required to run deuterium gradient shimming. If present, this module automatically holds the lock at its current value and switches the transmitter cable to pulse the lock coil when an experiment is run with `tn='lk'`. The module is strongly recommended for all users who wish to run deuterium gradient shimming in automation. See Table 53 for system configurations supported.

The system administrator must make a shimmap on deuterium before deuterium gradient shimming can be used. Follow the procedure "Mapping the Shims," on page 377, using the deuterium signal for all steps, (use `tn='lk'` for step 4, and select **Pfg H2** at step 6). The transmitter power (`tpwr`) should be kept low to avoid probe arcing, with a 90° pulse greater than about 200 µs.

The recommended parameters for different solvents are shown in Table 55.

The deuterium parameters are saved for future use when the shimmap is saved, and are used the next time gradient autoshimming is run.

Table 55. Deuterium Parameters

Solvent	*nt*	*d1 (sec)*	*Gain*	
			Inova	*Mercury*
deuterochloroform	8-32	3	36	18
dmso-d6	4-16	3	28	10
D_2O	1-4	3	24	6
deuterobenzene	1-4	3	24	6
deuteroacetone	1-4	6-12	24	6

Note: Actual parameters might vary, depending on solvent concentration, probe, and system hardware.

Homospoil Gradient Shimming for ^{1}H or ^{2}H

It is also possible to use the Z1 room temperature shim as a homospoil gradient, instead of using a pulsed field gradient or PFG. Use of this option is recommended only if a PFG amplifier or probe is not available. For details on how to configure a homospoil gradient, see the section “Homospoil Gradient Type” in the chapter on PFG modules operation in the manual *User Guide: Liquids NMR*. The system administrator must make a shimmap using homospoil before homospoil gradient shimming can be used. Follow the procedure in “Mapping the Shims,” on page 377.

At step 4 select `tn` as appropriate, set `tof` on resonance, and then find the 90° pulse. If deuterium is used, `tpwr` should be kept low, with a 90° pulse greater than about 200 µs.

At step 6, select either **Homospoil H1** for proton parameters or **Homospoil H2** for deuterium parameters, as appropriate. Homospoil gradients must be configured at this step (use `config` or set `gradtype='nnh'`).

At step 7, further testing of the gradient shimming parameters for homospoil can be done as follows:

1. Enter **`gmapsys`**, and click on **Set Params** > **Go,dssh**.
2. Using the 90° pulse from step 4, calibrate the 90° and 180° pulses to obtain an echo. Enter **`df`** to display the FID. You should see an echo forming in the middle of the FID.
3. If needed, adjust **`sw`** so that the gradient covers at least 10% of the spectral window. Increase **`np`** to 512 to improve Hz/point resolution. However, `np` should be adjusted so that `at` is not longer than the homospoil time limit (20 ms on standard UNITY*INOVA* and UNITY*plus* and 200 ms with the automated deuterium gradient shimming module). The acquisition time (`at`) should also be shorter than T_2. Set **`d2=at/4`**.

Once all the parameters are set, click on **Automake Shimmap** (step 8). The parameters are saved when the shimmap is done and are used the next time gradient autoshimming is run.

To use homospoil deuterium gradient shimming with different solvents, set the parameter **`gmap_findtof='y'`**. This should be done before making the shimmap, or may be set in the corresponding parameter set in `gshimlib/shimmaps`. Gradient shimming will then perform a calibration to find tof before autoshimming starts. This takes an extra iteration in

the shimming procedure, but is essential for homospoil deuterium gradient shimming to function correctly in automation.

Full Deuterium Gradient Shimming Procedure for Lineshape

The automated deuterium gradient shimming module must be installed to use this procedure.

1. Insert the appropriate lineshape sample (chloroform in acetone-d6) and find lock. Turn off spinning and disable sample changer control. Adjust lock power, lock gain, and lock phase as necessary. Do quick shimming on z1, z2, x1, y1 (use z1c, z2c, if present).
2. Find the 90° pulse on ^{2}H as follows:
   a. Enter **`s2pul tn='lk' tpwr=42 pw=200`**.
   b. Enter **`ga`** and wait for acquisition to finish. You should see only a single line. Place the cursor on resonance and enter **`nl movetof`**.
   c. Enter **`array('pw',20,100,100)`**. Enter **`ga`** and wait for acquisition to complete.
   d. Set **`pw90`** to the first maximum.
3. Set up gradient shim parameters. Enter **`gmapsys`** and click on **Set Params** > **Gradient, Nucleus**.
   a. If you have PFG, click on **Pfg H2**, set **`pw`** to one half the 90° pulse found in step 2 and set **`d1=6`**.
   b. If you have homospoil, click on **Homospoil H2**, set **`pw`** to the 90° pulse, **`p1`** to the 180° pulse, and **`d1=6`**.
4. Enter **`gmapsys`**, click on **Set Params** > **Go, dssh**, and wait for acquisition to complete. You should see two profile spectra.
5. Map the shims. Enter **`gmapsys`** and click on **Shim Maps** > **Automake Shimmap**. At the mapname prompt, enter a file name. Wait for acquisition to complete and the message to be displayed: `shimmap done!`
6. Perform shimming on z's. Enter **`gmapsys`** and click on **Autoshim on Z**. Wait for the acquisitions to complete, and the message to be displayed: `Gradient Autoshimming on Z done! N iterations`.
7. Enter **`acqi`** and adjust lock phase. Shim only on low-order nonspins (x1, y1, xz, yz, etc.). Do not shim on z's (z1, z2, etc.).
8. Perform shimming on z's. Repeat step 6.
9. Measure proton lineshape. Turn on spinner if appropriate, and enter **`acqi`** to make fine shim adjustment. Shim on all shims as necessary.

Setting Up Automation

You must have the deuterium gradient shimming module installed to perform deuterium gradient shimming in automation.

1. Find your 90° pulse on deuterium:
   - Enter **`s2pul tn='lk' tpwr=42 pw=200 gain=20`**.
   - Enter **`ga`**.

- Array `pw` to find the 90° pulse.

2. Set the parameters:
   - Enter **`gmapsys`**.
   - Click on **Set Params**.
   - Click on **Gradient**, **Nucleus**.
   - If you have pulsed field gradient, click on **Pfg H2** to select the parameter for deuterium with pfg. If you have homospoil gradients, select **Homospoil H2** instead.
   - Set `tpwr` and `pw` for 90° pulse and set `gain` as appropriate for your solvent.
   - Click on **Return**, then **Go, dssh** to test the parameters.

     You should see two top-hat profile spectra. Adjust `gain` and `nt` to see good signal-to-noise with no ADC overflow.

     The recommended parameters for different solvents are listed in Table 55.
3. Make a shimmap for a particular solvent using parameters for good signal-to-noise. If the solvent you most often use has a weak signal, make the map on a solvent with more signal. To make the map, click on **Shim Maps**, then **Automake Shimmap**. When prompted, enter a map name.
4. Test autoshimming by entering **`gmapsys`**, then click on **Autoshim on Z**.
5. When you are satisfied that autoshiming works well for your particular solvent, open a shell window and edit the `gmapz` macro. Go to the bottom of the macro and uncomment the section appropriate for your solvent (the solvent on which the shimmap was made). Use parameters as in step 2. Repeat step 2 for all solvents of interest.
6. Edit the `setlk` macro and uncomment the section setting `lockpower`, `lockgain`, and `z0` for different solvents (you must first determine the appropriate values for different solvents).
7. Change the appropriate parameter sets in `/vnmr/stdpar` to use `wshim='g' spin='y' spin=20` (or another appropriate spin value). Use `alock='y'` if you use the `setlk` macro.

Note: Use `config` to set the gradient type to Homospoil or PFG for use in automation. Setting `gradtype` from the command line does not set the gradient type for automation.

Suggestions for Improving Results

Calibrate the 90° pulse and adjust `tpwr`, `pw`, and `gain` to optimize signal-to-noise. Reduce gain if ADC overflow occurs, which may appear as wings on the profile.

For solvents with long T_1, set **`d1`** to 3 to 5 times T_1, or use a small flip angle for `pw`. Stimulated echoes may otherwise result, which may appear as excess noise or a beat pattern in the spectrum, or as secondary echoes in the FID (use `df` to observe this).

The phase encode delay `d3` is arrayed to two values, the first of which is zero. The second value can be increased for better signal-to-noise in the phase maps, up to about the point where the amplitude of the second profile is half that of the first (about 2/3 T_2 without radiation damping; radiation damping can be severe in water ^{1}H). However, longer `d3` values increase the phase excursion, and can make it difficult to shim large shim corrections (especially Z1). Typical ^{1}H values are 5 to 30 ms, and typical ^{2}H values are 30 to 200 ms.

If the shims are far off when making a shimmap, the second value of d3 might be too small. If this problem occurs, decrease the second value of d3 to temporarily one-half to one-quarter its value.

When reinstalling a probe, make sure it is in the same vertical position in the magnet barrel as when the shimmap was made. If you are unsure, make a new shimmap, which typically takes only a few minutes.

Alternate between z-axis gradient shimming and shimming the low-order x- and y-axis shims by other methods (e.g., on lock level). The z-axis shims account for the majority of sample volume changes (changes in height), and the x- and y-shims are relatively insensitive to change in height. Evaluate shimming for a particular application, since the ideal lineshape may vary with the application.

The high-order shims can sometimes be set off-scale during shimming. This may occur if the sample is short, or if the sample is improperly seated in the probe, or if the high-order shims are weak or other effects. In such cases, the off-scale shim is set to maximum, and shimming continues with lower-order shims. Superior results can be obtained in some cases by first setting **`gzsize=4`** and clicking on Autoshim on Z to shim on `z1`–`z4`, and then shimming the low-order transverse shims, and then increasing `gzsize` and shimming again. This may also be done using `gmap_z1z4='y'`. On a short sample it also can be useful to remap the shims.

Some shim systems may need additional time when running the shim mapping experiment to allow the shims to settle. The added time is especially noticeable on some systems for Z4. To account for added time, lengthen the `d1` delay or add dummy scans in between each array element (e.g., `ss=-2`). Decreasing the amount a shim is offset also allows the shim to settle more quickly. Enter `gmapsys('vi')` to edit the values in the Offset column, and then enter `gmapsys('shimmap','manual')` to map the shims with user-defined offsets. A new mapname may also be set using `gmapsys('vi')`.

Coarse shims are used on systems on which they are available. To use fine shims on these systems, enter `gmapsys('vi')` to edit the entries in the shim column (e.g., change `z1c` to `z1`), and then enter `gmapsys('shimmap','manual')` to map the shims.

For samples in H_20, the water protons provide sufficient signal for shimming. For samples other than water, deuterium gradient shimming is strongly recommended if there is sufficient deuterium signal. Proton gradient shimming can be made to work in samples other than water if there is sufficient proton signal and the signal is well-resolved (does not overlap with other strong resonances). Gradient shimming can also be done on a water sample of equal height of the sample of interest, and then the sample of interest can be inserted.

For further information, refer to the entries for `gmapshim`, `gmapsys`, and `gmapz` in the *VNMR Command and Parameter Reference*.

Gradient Shimming Menus

The top-level gradient shimming menu is the Gradient Shimming System menu, which is opened by entering `gmapsys`.

From this menu and submenus, there are buttons to open the following menus:

- Gradient Shimming Setup menu

- Gradient Nucleus Parameter Setup menu

- Gradient Shimming Map menu

- Gradient Shimming Files menu

- Gradient Shim Setting menu

- Gradient Shimming Display menu

- Gradient Shimming Plot menu

Two other gradient shimming menus are available for the general user:

- Automated Shimming menu. To open, click on Main Menu | Setup | Shims.

- Gradient Autoshimming Files menu. To open, enter `gmapshim('files')`.

A description of each menu (in the same order as above), the button or command for opening the menu, and the action of each button in the menu is given in the following sections. On all menus, the left mouse button is used to click on the desired choice in the menu. The other mouse buttons are not active.

Gradient Shimming System Menu

The Gradient Shimming System menu is the top-level menu for gradient shimming setup and shim mapping. To open this menu, enter `gmapsys`.

Button	*Description*
Set Params	Enter Gradient Shimming Setup menu.
Shim Maps	Enter Gradient Shimming Map menu.
Autoshim on Z	Start gradient shimming using current parameters. Fit is displayed with each iteration.
Set Shims	Enter Gradient Shim Setting menu.
Display	Enter Gradient Shimming Display menu.
Quit	Return to previous experiment.

Gradient Shimming Setup Menu

The Gradient Shimming Setup menu is used for calibrating gradient shimming parameters. To open this menu, click on Set Up in the Gradient Shimming System menu.

Button	*Description*
Go, dssh	Run and display profile spectra.
Gradient,Nucleus	Enter Gradient,Nucleus parameter setup menu.
Find gzwin	Run a calibration experiment to set parameters `tof` and `gzwin`, to optimize the spectral window for a given `gzlvl`. Pulse length and gain should be correctly set before using this button.
Calculate gzwin	Determine `tof` and `gzwin` from the profile spectrum using cursor positions.
Find tof	Run a calibration experiment to set tof. Pulse length and gain should be correctly set before using this button
Add Params	Add parameters for gradient shimming.
Return	Return to the Gradient Shimming System menu.

Gradient Nucleus Parameter Setup Menu

The Gradient Nucleus Parameter Setup menu is used for setting parameters according to type of gradient and nucleus. Recabling may be required to observe ^{1}H or ^{2}H nucleus. Check the value of `gradtype` when done. To open this menu, click on **Gradient,Nucleus** in the Gradient Shimming Setup menu.

Button	*Description*
Pfg H1	Set parameters for pulsed field gradient on ^{1}H.
Pfg H2	Set parameters for pulsed field gradient on ^{2}H.
Homospoil H1	Set parameters for homospoil gradient on ^{1}H.
Homospoil H2	Set parameters for homospoil gradient on ^{2}H.
Return	Return to the Gradient Shimming Setup menu.

Gradient Shimming Map Menu

The Gradient Shimming Map menu is used for making and retrieving shimmaps. To open this menu, click on **Shim Maps** in the Gradient Shimming System menu.

Button	*Description*
Automake Shimmap	Find `gzwin` and then run shimmap experiment.
Make Shimmap	Run shimmap experiment.
Shimmap Files	Enter Gradient Shimming Files menu.
Current Mapname	Show current mapname used for gradient shimming.
Save As	Save current parameter set and shimmap files. Enter mapname when prompted.
Return	Return to the Gradient Shimming System menu.

Gradient Shimming Files Menu

The Gradient Shimming Files menu is used for loading and copying shimmaps. To open this menu, click on **Shimmap Files** in the Gradient Shimming Map menu.

Button	*Description*
Cd to Userdir or	Change directory to `userdir` shimmap.
Cd to Systemdir	Change directory to `systemdir` shimmap.
Copy to Userdir or	Copy selected shimmap into user shimmap directory.
Copy to Systemdir	Copy selected shimmap into system shimmap directory.
Load Shimmap	Load files for selected shimmap.
Load Shimmap & Params	Load files and parameters for selected shimmap.
Rename	Rename selected shimmap under a new mapname.
Return	Return to the Gradient Shimming Map menu.

Gradient Shim Setting Menu

The Gradient Shim Setting menu sets shim values. To open this menu, click on **Set Shims** in the Gradient Shimming System menu.

Button	*Description*
Old Shims	Set shims from before first iteration of gradient shimming.
New Shims	Set shims from last iteration of gradient shimming.
Min Shims	Set shims from minimum rms err in last set of gradient shimming iterations.
Return	Return to the Gradient Shimming System menu.

Gradient Shimming Display Menu

The Gradient Shimming Display menu is used for displaying items associated with gradient shimming. To open this menu, click on **Display** in the Gradient Shimming System menu.

Button	*Description*
dgs	Display shim parameters.
List Shims	Display current mapname and last shim changes.
Display Shimmap	Display current shimmap used by gradient shimming.
Display Fit	Display shim fit from last iteration of gradient shimming.
Show Record	Display record of shim adjustments from last gradient shimming run.
Plot	Enter Gradient Shimming Plot menu.
Return	Return to the Gradient Shimming System menu.

Gradient Shimming Plot Menu

The Gradient Shimming Plot menu is used for printing and plotting shim parameters and files. To open this menu, click on **Plot** in the Gradient Shimming Display menu.

Button	*Description*
Print dgs	Print shim parameters.
Print Shims	Print current mapname and last shim changes.
Plot Shimmap	Plot current shimmap.
Plot Fit	Plot last iteration shim fit.
Plot b0	Plot `b0` shim plot.
Return	Return to the Gradient Shimming Display menu.

Automated Shimming Menu

The Automated Shimming menu is used for running automated gradient shimming after a shimmap is made. To open this menu, click on **Main Menu** | **Setup** | **Shim**.

Button	*Description*
Lock Autoshim	Start lock autoshim using current `method`.
Lock Autoshim z1z2	Start lock autoshim on z1z2.
Gradient Autoshim on Z	Start gradient autoshim on z-axis shims
Return	Return to Acquisition Parameter Setup menu.

Gradient Autoshimming Files Menu

The Gradient Autoshimming Files menu is used for loading shimmaps. To open this menu, enter `gmapshim('files')`.

Button	*Description*
Cd to Userdir or	Change directory to `userdir` shimmaps.
Cd to Systemdir	Change directory to `systemdir` shimmaps.
Current Mapname	Show current mapname used for gradient shimming.
Load Shimmap	Load files and parameters for selected shimmap.
Load Shimmap & Params	Load files and parameters for selected shimmap.
Rename	Rename selected shimmap under a new mapname.
Return	Return to Main menu.

Chapter 12. PFG Modules Experiments

Sections in this chapter:

- 12.1 “GCOSY—PFG Absolute-Value COSY,” this page
- 12.2 “GHMQC—PFG HMQC,” page 394
- 12.3 “GHMQCPS—PFG HMQC, Phase Sensitive,” page 394
- 12.4 “GHSQC—PFG HSQC, Absolute Value or Phase Sensitive,” page 395
- 12.5 “GMQCOSY—PFG Absolute-Value MQF COSY,” page 396
- 12.6 “GNOESY—PFG NOESY,” page 397
- 12.7 “GTNNOESY—PFG TNNOESY,” page 398
- 12.8 “GTNROESY—PFG Absolute-Value ROESY,” page 398
- 12.9 “PFG Selective Excitation,” page 400

This chapter describes pulse sequences used with Varian PFG (Pulsed Field Gradient) modules. Each pulse sequence has a macro on the system that converts a 1D S2PUL parameter set into a parameter set ready to run the PFG experiment named.

The exact numbers used for gradient levels depends on the gradient amplifier and probe. For this reason, values listed are expressed in terms of gauss/cm.

12.1 GCOSY—PFG Absolute-Value COSY

The `gcosy` macro sets up parameters for the pulse sequence GCOSY (Pulsed Field Gradient Absolute-Value Correlated Spectroscopy). GCOSY is available on all systems with the PFG module.

Parameters

Table 56 lists the parameters used in GCOSY.

Table 56. GCOSY Parameters

`qlvl`	1 (quantum selection level)
`gzlvl1`	Gradient amplitude (–32768 to +32767; use 3 gauss/cm)
`grise`	Gradient rise and fall time (in seconds, 0.00001)
`gstab`	Optional delay for stability (in seconds)
`gt1`	Gradient duration (in seconds, 0.002)
`phase=1`	Selects echo N-type coherence selection (default)
`phase=2`	Selects anti-echo P-type coherence selection

Processing

Process N-type data with `wft2d(1,0,0,1)`. Process P-type data with `wft2d(1,0,0,-1)`. The optional `'t2dc'` argument to `wft2d` can be useful.

12.2 GHMQC—PFG HMQC

The `ghmqc` macro sets up parameters for the pulse sequence GHMQC (Pulsed Field Gradient Heteronuclear Multiple-Quantum Correlation). GHMQC is available on all systems with the PFG module.

Parameters

Table 57 lists the parameters used in GHMQC.

Table 57. GHMQC Parameters.

`grise`	Gradient rise and fall time (in seconds; 0.00001).
`gstab`	Optional delay for stability (in seconds).
`gt1`	Gradient duration (in seconds, 0.001).
`gt2`	Gradient duration (in seconds, 0.001).
`gt3`	Gradient duration (in seconds, 0.001).
`gzlvl1*`	Gradient amplitude (–32768 to +32767).
`gzlvl2*`	Gradient amplitude (–32768 to +32767).
`gzlvl3*`	Gradient amplitude (–32768 to +32767).
`j`	1JXH (in Hz, 140 typical for ^{1}H-^{13}C).
`pwx`	Decoupler pulsed `pw90`.
`pwxlvl`	Decoupler pulse power level.
* For more information, see the text in the section that contains this table.	

`gzlvl1`, `gzlvl2`, and `gzlvl3` and their times (`gt1`, `gt2`, and `gt3`) can eventually be fixed in their relationship (i.e., `2:2:-1`, `0:4:-3`, etc.).

For ^{13}C, try `gzlvl1=10` (gauss/cm), `gt1=0.002`, `gzlvl2=10` (gauss/cm) `gt2=0.00`, `gzlvl3=5` (gauss/cm), `gt3=0.002`, array `gzlvl3` for maximum signal.

Processing

If `gzlvl3` is the same sign as `gzlvl1` and `gzlvl2` (N-type data), process with `wft2d('t2dc')`.

If `gzlvl3` is opposite in sign to `gzlvl1` and `gzlvl2` (P-type data), process with `wft2d('t2dc','ptype')`. The sequence sets three gradients, all separately.

12.3 GHMQCPS—PFG HMQC, Phase Sensitive

The `ghmqcps` macro sets up parameters for GHMQCPS (Pulsed Field Gradient HMQC, Phase-Sensitive Version). Note that GHMQCPS does not do gradient HMBC. Features of this pulse sequence include:

- Two `gt1`'s precede and follow `d2`, instead of follow and precede X 90's.
- TANGO pulse followed by a homospoil gradient precede the actual sequence.

- `sspul` option included.
- FAD on both first X 90 and first X 180.
- Two-step phase cycling on X 90's.

GHMQCPS is available on all systems, except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, with the PFG module.

Processing

Use `wft2d(1,0,1,0,0,1,0,-1)` with `rp` and `lp` the same as in `s2pul` spectrum. `rp1` and `lp1` will be close to zero. (`wft2dnp` can also be used with the "usual" 45° shift in f1 and f2).

Recommendations

For small molecule in H_2O and ^{13}C:

```
gzlvl1=10000
gt1=0.001
gzlvl3=5025
gt3=0.001
gstab=0.0005
hsgpwr=10000
hsgt=0.005
```

`nt=1 per inc.` (FADed axials, though substantially reduced compared to original `ghmqcps`).

`nt=2 per inc.` (no significant axials or FADed axials even with high concentration samples, recommended).

`nt=4 per inc.` (more improvement in axial suppression).

12.4 GHSQC—PFG HSQC, Absolute Value or Phase Sensitive

The `ghsqc<(nucleus)>` macro sets up parameters for the pulse sequence GHSQC (Pulsed Field Gradient Heteronuclear Single-Quantum Correlation), absolute value or phase sensitive. The optional argument `nucleus` is ^{13}C or ^{15}N (e.g., `ghsqc(15N)`). GHSQC is available on all systems, except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, with the PFG module.

Parameters

Table 58 lists the parameters used in GHSQC.

Gradients need to maintain an approximately 4:1 (^{13}C) or 10:1 (^{15}N) ratio. The exact value can be determined from a ratio of the gyromagnetic ratios of the nuclei. The ratio can be controlled with either amplitude or time.

For ^{13}C, try `gzlvl1=20` (gauss/cm), `gt1=0.002`, `gzlvl2=20` (gauss/cm), `gt2=0.0005`.

For ^{15}N, try `gzlvl1=30` (gauss/cm), `gt1=0.0025`, `gzlvl2=15` (gauss/cm), `gt2=0.0005`.

Table 58. GHSQC Parameters

`bigt`	Constant time for X evolution (try 3 milliseconds).
`dmf`	Decoupler modulation frequency for 1st decoupler; controlled by `dpwr`.
`dpwr`	Power level for decoupling.
`f1180`	y/n flag for 1/2 dwell starting t_1 evolution delay.
`gt1`	First gradient duration.
`gt2`	Second gradient duration.
`gzlvl1*`	First gradient power level.
`gzlvl2*`	Second gradient duration.
`j`	One-bond heteronuclear coupling constant (140 for ^{13}C, 90 for ^{15}N).
`nt`	Number of transients; works with `nt=1`, but 2 or higher improves data.
`phase=1`	Use to generate an absolute-value data set.
`phase=1,2`	Use to select N,P-type selection (to be sorted later).
`pwx`	Pulse width for hard decoupler pulses.
`pwxlvl`	Decoupler power level for hard decoupler pulses.
`satmode`	Saturation mode; `'y'` for transmitter presaturation.
`satdly`	Presaturation delay used if `satmode='y'`.
`satfrq`	Frequency desired for presaturation.
`satpwr`	Presaturation power.
* For more information, see the text in the section that contains this table.	

Processing

Use `wft2d('t2dc',1,0,1,0,0,1,0,-1)` for `phase=1,2` (phase up the first increment). The `t2dc` argument is optional. If the peaks appear to be reversed along the F_1 axis, multiply the last half of the coefficients by `-1` to produce `wft2d(1,0,1,0,0,-1,0,1)`. This sequence depends on the sign of the gradients used and on the order of phase (`phase=1,2` versus `phase=2,1`).

Use `wft2d(1,0,0,1)` for `phase=1`.

12.5 GMQCOSY—PFG Absolute-Value MQF COSY

The `gmqcosy` macro sets up parameters for the pulse sequence GMQCOSY (Pulsed Field Gradient Multiple-Quantum Filtered Correlated Spectroscopy). GHMQOSY is available on all systems with the PFG module.

Parameters

Table 59 lists the parameters used in GMQCOSY.

Gradient levels for organic samples: try `gzlvl1=10` (gauss/cm), `gt1=0.003`. Gradient levels for H2O samples: try `gzlvl1=10` (gauss/cm), `gt1=0.008`

Processing

Process N-type data with `wft2d(1,0,0,1)`. Process P-type data with `wft2d(1,0,0,-1)`. The optional `'t2dc'` argument to `wft2d` can be useful.

Table 59. GMQCOSY Parameters

`qlvl`	2 (quantum selection level for DQF COSY).
`grise`	Gradient rise and fall time (in seconds, 0.00001).
`gstab`	An Optional delay for stability (in seconds).
`gt`	Gradient duration (in seconds, 0.001).
`gzlvl1*`	Gradient amplitude (use 10 to 15 gauss/cm because it is multiplied by `qlvl+1`).
`phase=1`	Selects echo N-type coherence selection (this is the default)
`phase=2`	Selects anti-echo P-type coherence selection.
* For more information, see the text in the section that contains this table.	

12.6 GNOESY—PFG NOESY

The `gnoesy` macro sets up parameters for the pulse sequence GNOESY (Pulsed Field Gradient Nuclear Overhauser Effect Spectroscopy), absolute value or phase sensitive. GNOESY is available on all systems with the PFG module.

Parameters

Table 60 lists the parameters used in GNOESY.

Table 60. GNOESY Parameters

`grise`	Gradient rise and fall time (in seconds; 0.00001).
`gstab`	Optional delay for stability (in seconds).
`gt1`	Gradient duration (in seconds, 0.003).
`gt2`	Gradient duration (in seconds, 0.012).
`gt3`	Gradient duration (in seconds, 0.003).
`gzlvl1*`	Gradient amplitude (–32768 to +32767; use 30 gauss/cm).
`gzlvl2*`	Gradient amplitude (–32768 to +32767; use 30 gauss/cm).
`gzlvl3*`	Gradient amplitude (–32768 to +32767; use 30 gauss/cm).
`phase=1`	Selects echo N-type coherence selection (`phase=1` is default);
`phase=2`	Selects antiecho P-type coherence selection;
`phase=1,2`	Selects phase-sensitive acquisition (N,P).
* For more information, see the text in the section that contains this table.	

For organic samples, try `gzlvl=gzlvl2=gzlvl3=10` (gauss/cm), `gt1=gt3=0.003`, `gt2=0.012`.

For H_2O samples, try `gzlvl1=gzlvl2=gzlvl3=30` (gauss/cm), `gt1=gt3=0.003`, `gt2=0.012`.

Processing

Process N-type data with `wft2d(1,0,0,1)`; process P-type data with `wft2d(1,0,0,-1)`. The optional `'t2dc'` argument to `wft2d` can be useful. Process phase-sensitive data (`phase=1,2`) with `wft2d(1,0,0,1,0,1,1,0)`.

12.7 GTNNOESY—PFG TNNOESY

The `gtnnoesy` macro sets up parameters for the pulse sequence GTNNOESY (Pulsed Field Gradient TN Nuclear Overhauser Effect Spectroscopy), absolute value or phase sensitive (includes optional presaturation). GTNNOESY is available on all systems, except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, with the PFG module.

Parameters

Table 61 lists the parameters used in GTNNOESY.

Table 61. GTNNOESY Parameters

`grise`	Gradient rise and fall time (in seconds; 0.00001).
`gstab`	Optional delay for stability (in seconds).
`gt1`	Gradient duration (in seconds, 0.003).
`gt2`	Gradient duration (in seconds, 0.012).
`gt3`	Gradient duration (in seconds, 0.003).
`gzlvl1`	Gradient amplitude (–32768 to +32767; use 30 gauss/cm).
`gzlvl2`	Gradient amplitude (–32768 to +32767; use 30/gauss/cm).
`gzlvl3`	Gradient amplitude (–32768 to +32767; use 30 gauss/cm).
`phase=1`	Selects echo N-type coherence selection (`phase=1` is the default);
`phase=1,2`	Selects phase sensitive acquisition (N,P).
`phase=2`	Selects antiecho P-type coherence selection;
`satdly`	Length of saturation during relaxation delay.
`satmode`	Saturation mode. Use analogously to `dm` (i.e., `satmode='nnn'`, `satmode='ynn'`, or `satmode='yyn'` (recommended)).
`satpwr`	Power level for solvent saturation

For organic samples, try `gzlvl1=gzlvl2=gzlvl3=10` (gauss/cm), `gt1=gt3=0.003`, `gt2=0.012`.

For H_2O samples, try `gzlvl1=gzlvl2=gzlvl3=30` (gauss/cm), `gt1=gt3=0.003`, `gt2=0.012`.

Processing

Process N-type data with `wft2d(1,0,0,1)`. Process P-type data with `wft2d(1,0,0,-1)`. The optional `'t2dc'` argument to `wft2d` can be useful.

Process phase-sensitive data (`phase=1,2`) with `wt2d(1,0,0,1,0,1,1,0)`.

12.8 GTNROESY—PFG Absolute-Value ROESY

The `gtnroesy` macro sets up parameters for GTNROESY pulse sequence (including optional presaturation). GTNROESY is available on all systems, except *MERCURY-VX*, *MERCURY*, and *GEMINI 2000*, with the PFG module.

Parameters

Table 62 lists the parameters used in GTNROESY.

Table 62. GTNROESY Parameters

d2corr*	Empirical correction, in µs, of d2 (dependent on effective field of spin lock, i.e., tpwr and/or ratio).
grise	Gradient rise and fall time (in seconds, 0.00001).
gstab	Optional delay for stability (in seconds).
gt1	Gradient duration (in seconds, 0.0015).
gt2	Gradient duration (in seconds, 0.0015).
gzlvl1	Gradient amplitude (–32768 to +32767; use 3 gauss/cm).
gzlvl2	Gradient amplitude (–32768 to +32767; use 3 gauss/cm).
mix	Mixing time.
nt	Set to 1 (min.), multiple of 2 (max.), multiple of 8 (recommended).
p1	90° pulse on protons (power level at p1lvl).
p1lvl	Power level for the p1 pulse.
phase=1	Selects P-type coherence selection (phase=1 is the default);
phase=2	Selects N-type coherence selection.
phase=1,2	For f_1 quadrature by hypercomplex method (uses f_1 axial peak displacement)
phase=3	For f_1 quadrature by the TPPI method.
pw	Small (30°) pulse on protons (active only if ratio > 0). If pw=0, pw is set to p1/3
ratio	tau/pw (uses CW transmitter lock if ratio is zero).
rocomp='n'	Sets no resonance offset compensation.
rocomp='y'	Sets resonance offset compensation (recommended).
satdly	Length of saturation during relaxation delay.
satmode	Saturation mode. Use analogously to dm (i.e., satmode='nnn', satmode='ynn', or satmode='yyn' (recommended)).
satpwr	Power level for solvent saturation
sspul='y'	Selects for trim(x)-trim(y) sequence at start of pulse sequence.
tpwr	Power level for the spin lock pulse(s).
* For more information, see the text in the section that contains this table.	

d2corr can be determined from the lp1 and sw1 values of a properly phased spectrum by d2corr=(lp1*1e6)/(360*sw1). Note that d2corr seems to be dependent on sw1. They are actually independent because changes in sw1 result in corresponding changes in lp1, which makes their ratio constant.

Do the following steps to find d2corr so that lp1=0, which gives better baselines in f_1:

1. Run a gtnroesy experiment with d2corr set either at 0 or at a value found previously (nt and ni can be smaller, and the spectrum can be transformed early to do step 2).
2. Phase the resulting spectrum in f_1. Determine lp1 and calculate d2corr from the relationship d2corr=(lp1*1e6)/(360*sw1).
3. Add this value to the value of d2corr used in step 1.
4. Rerun the experiment—lp1 should be close to zero.
5. Note this value for any future experiment with the same value of tpwr and ratio.

Use of any method to make lp1=0 will result in a dc offset of f_1 slices. This should be removed by dc2d('f1') after the 2D transform. Enough noise should be left on the edges (in f_1) to permit this dc correction.

Processing

Process N-type data with `wft2d(1,0,0,1)`; process P-type data with `wft2d(1,0,0,-1)`. The optional `'t2dc'` argument to `wft2d` can be useful.

12.9 PFG Selective Excitation

The `selexcit` macro sets up parameters for PFG selective excitation with a `presat` option. Selective excitation is based on double PFG spin-echo, also known as excitation sculpting. Available on all systems, except *GEMINI 2000*, with the PFG module.

Parameters

Table 63 lists parameters used in PFG selective excitation.

Table 63. Selective Excitation Parameters

`gt1`	Echo gradient during DPFGSE; typical value is `0.00025`.
`gt2`	Echo gradient during DPFGSE; typical value is `0.0005`.
`gzlvl1`	Echo gradient during DPFGSE; typical value is `8000`.
`gzlvl2`	Echo gradient during DPFGSE; typical value is `5000`.
`hsgpwr`	Homospoil gradient during DPFGSE.
`pwshape`	Selective proton, shape; values are typically created by `Pbox`.
`selpw`	Selective proton, pulse width; values are typically created by `Pbox`.
`selpwr`	Selective proton, 180 power; values are typically created by `Pbox`.

Presaturation option is included (using `satflg`, `satdly`, `satfrq`, and `satpwr`).

Reference

Shaka, et.al *J. Am. Chem. Soc.* **1995**, *117*, 4199.

Chapter 13. LC-NMR Accessory Operation

Sections in this chapter:

- 13.1 “LC-NMR Experiments Overview,” page 402
- 13.2 “Solvent Suppression in LC-NMR,” page 404
- 13.3 “Maximizing LC Sensitivity,” page 410
- 13.4 “On-Flow LC-NMR Experiments,” page 410
- 13.5 “Stop-Flow LC-NMR Experiments,” page 414
- 13.6 “Time-Slice Stop-Flow Experiments,” page 421
- 13.7 “2D LC-NMR Experiments,” page 425
- 13.8 “Analyte Collector Operation,” page 427
- 13.9 “Data Processing Methods,” page 429
- 13.10 “Delay Time Calibration,” page 431
- 13.11 “Chromatography for LC-NMR,” page 435
- 13.12 “Cascade LC-NMR Stop-Flow Program,” page 443

The Varian LC-NMR system comprises an HPLC system, a Microflow probe, an NMR spectrometer, and an interface that coordinates the operation of the HPLC and NMR systems. Figure 113 shows a block diagram of the LC-NMR system.

This chapter provides overviews of the software as well as descriptions of the two types of LC runs—on-flow and stop-flow. Also in this chapter are procedures that outline different methods of running on-flow and stop-flow experiments.

The software used with the LC-NMR system is composed of two main parts:

- *Cascade LC program* – Runs on an IBM-compatible PC with a Data Translation 16-bit ADC board and Microsoft Windows 95 operating system. Cascade interprets signals from the HPLC detector analog output and from the NMR system to start and stop flow through the HPLC system and the Microflow probe for stop-flow and on-flow experiments. For a description of the windows and buttons in the Cascade program, refer to “Cascade LC-NMR Monitor Window,” page 445.
- *LC software module for VNMR* – Consists of a variety of components, including pulse sequences, pulse shapes, data acquisition macros, data processing macros, and assorted other macros. Highly sophisticated solvent suppression can be run using the VNMR LC-NMR DG panes, the command line, or the LC-NMR menu system. Procedures in this chapter describe using the LC-NMR DG panes.

You might also need to use the STAR Chromatography software for certain setup procedures and calibrations. For information on using STAR Chromatography, see the procedures in “Chromatography for LC-NMR,” page 435.

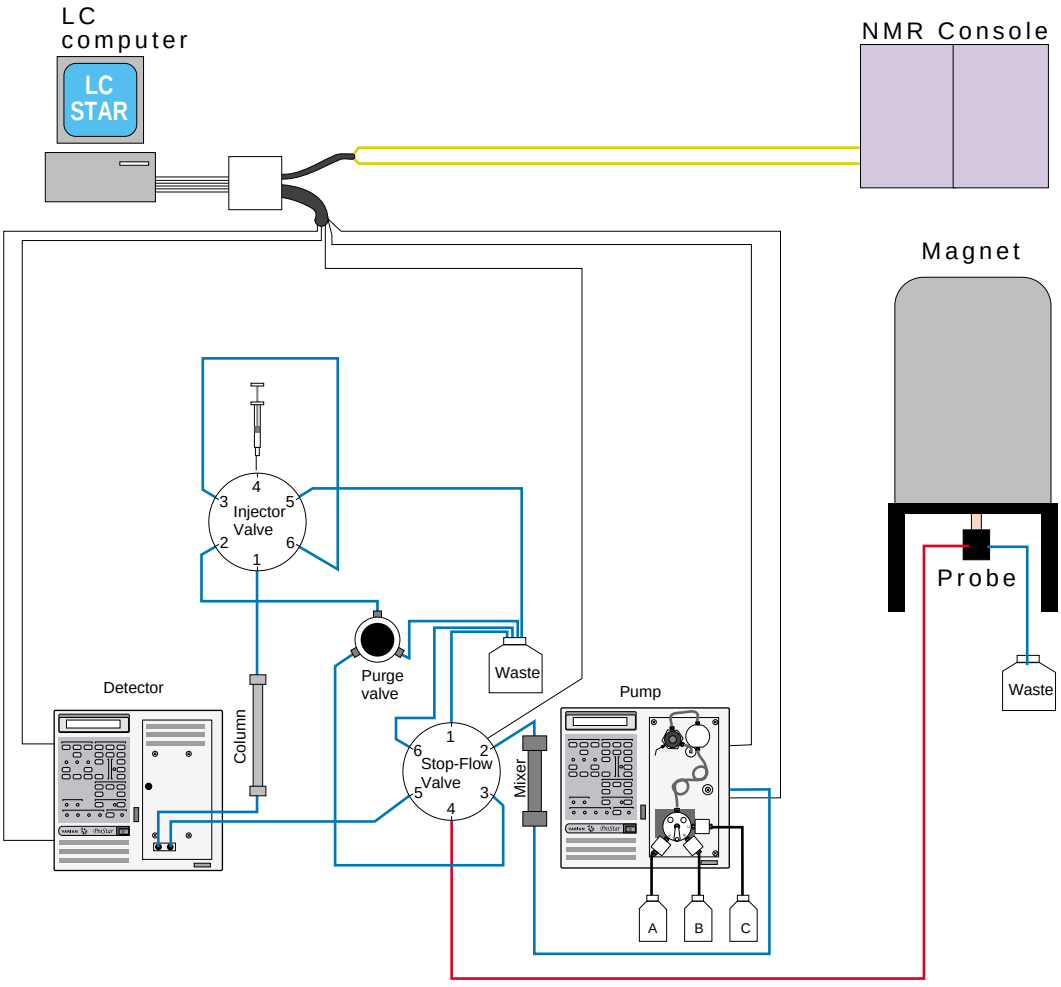


Figure 113. LC-NMR Block Diagram

13.1 LC-NMR Experiments Overview

Before you can start an LC run, the LC hardware must be set up, cables and plumbing connected, and the Microflow probe installed. Also, the probe must be tuned and any touch-up shimming should be done.

The following steps outline the basic protocol of the LC-NMR experiment process:

1. Retrieve a parameter set appropriate for LC-NMR by using either the menu system (**Setup** -> **Flow-NMR**-> **Initialize LC-NMR**) or through the **`rtp`** command.
2. Tune the probe and adjust shimming if necessary.
3. Make sure PFG is working and on (`pfgon`).
4. Make sure the **Cascade LC program** is running on the PC and set for the type of run (on-flow or stop-flow) that you wish to make (refer to "Cascade LC-NMR Stop-Flow Program," page 443).

   Even for on-flow experiments, the Cascade program should be running on the PC, because all signal propagation between the NMR spectrometer and the HPLC system is handled by the this program.

Running Cascade with the stop-flow run type and checking the **Inhibit Apex Detection** checkbox gives you the same effect as running an on-flow run type while preserving the ability to override the system and stop on a signal of interest.

5. If your LC pump is not already pumping solvent, push the **PUMP** button on the pump and allow the pressure to stabilize.
6. Set the NMR experimental conditions.
   - If you are using solvent suppression, follow the steps in "Setting Up Solvent Suppression," page 404.
   - If you are not using solvent suppression, make sure the ^{1}H and ^{13}C 90° pulse width values are determined.
7. Determine the delay time between the HPLC system and the Microflow probe, as described in "Delay Time Calibration," page 431.
8. Run the desired LC-NMR experiment:
   - For isocratic or gradient on-flow experiments, go to "On-Flow LC-NMR Experiments," page 410.
   - For manual or automated stop-flow experiments, go to "Stop-Flow LC-NMR Experiments," page 414.
   - For manual or automated time-slice stop-flow experiments, go to "Time-Slice Stop-Flow Experiments," page 421.
   - For manual or automated 2D LC-NMR experiments, go to "2D LC-NMR Experiments," page 425.
   - For analyte collection or analysis with the Analyte Collector option, go to "Analyte Collector Operation," page 427.

If you wish, monitor the progress of the run with the Cascade program. You can intervene if needed.

Note that in stop-flow experiments you can override any time-programmed or automatic setting by entering in a new value, clicking on a button, or toggling a checkbox.

If you observe peaks on the Chromatogram display in the Cascade program that do not meet the detection criteria set for the Cascade program, you can use the Stop Flow buttons to halt the system and activate the stop flow valve.

13.2 Solvent Suppression in LC-NMR

Solvent suppression is invariably the first step needed for any LC-NMR experiment. After the initial solvent suppression setup is done, however, you only need to click the Trial WET button in the LC-NMR pane to perform further optimizations.

This section contains the following:

- "Setting Up Solvent Suppression," page 404
- "Troubleshooting Solvent Suppression," page 408
- "Evaluating Solvent Mixture Equilibration," page 408
- "Solvent Suppression: Background Information," page 409

Setting Up Solvent Suppression

1. Retrieve a parameter set appropriate for LC-NMR by using either the menu system (**Setup** -> **Flow-NMR** -> **Initialize LC-NMR**) or through the **`rtp`** command.
2. Regulate the probe temperature (preferably to a value close to ambient). The value of `temp` is displayed in the Acq & Obs pane.
3. Turn the lock off. Set **`lockgain`** and **`lockpower`** to zero.

   Turning off lock eliminates *lock pull* during each spectrum of a solvent gradient experiment and *lock jumps*. *Lock pull* is caused by the constantly changing HOD frequency during the signal averaging. *Lock jumps* occur if the concentration of the deuterated species drops below the detection threshold (see the Noise reject parameter).

   The sudden loss of lock, with its commensurate sudden change in field, is annoying during Trial WET setup operations. Even more critically, it can disrupt solvent suppression during an LC-NMR run. Although turning the lock off is not mandatory, data is measurably better unless you are running a long stop-flow experiment.
4. Tune the probe and adjust shimming if necessary.
5. Check if the global parameters `ref_pw90` and `ref_pwr` exist:

   `ref_pw90?`
   `ref_pwr?`

   or **click** on the **Sequence tab** and look at the **lc1d Sequence pane**, see Figure 114:
6. If they do not exist, create them:

   ```
   create('ref_pw90','pulse')
   create('ref_pwr','real')
   ```
7. If they do exist, make sure they are set correctly for the probe being used (e.g., `ref_pwr=44` and `ref_pw90=25`). The `ref_pwr` used must be within the linear portion of the amplifier's operational range. These values are used to calculate the power levels for the shaped pulses.
8. **Click** on the **LCNMR/STARS tab**, see Figure 115, to open the **LC-NMR pane**.
9. **Click** on **WET** and **select: Solv 1**, **Solv 2**, **Solv 3**, or **Solv 4** to set the number of NMR signals (up to 4 signals using this pane see step 13 for suppression of up to 7 signals) you intend to suppress.

   The first NMR signal to be suppressed must be suppressed using Solv 1. If the spectrum contains two or more NMR signals that are to be suppressed, select Solv 2, Solv 3, or Solv 4 until the appropriate number of signals have been chosen. Solv 2

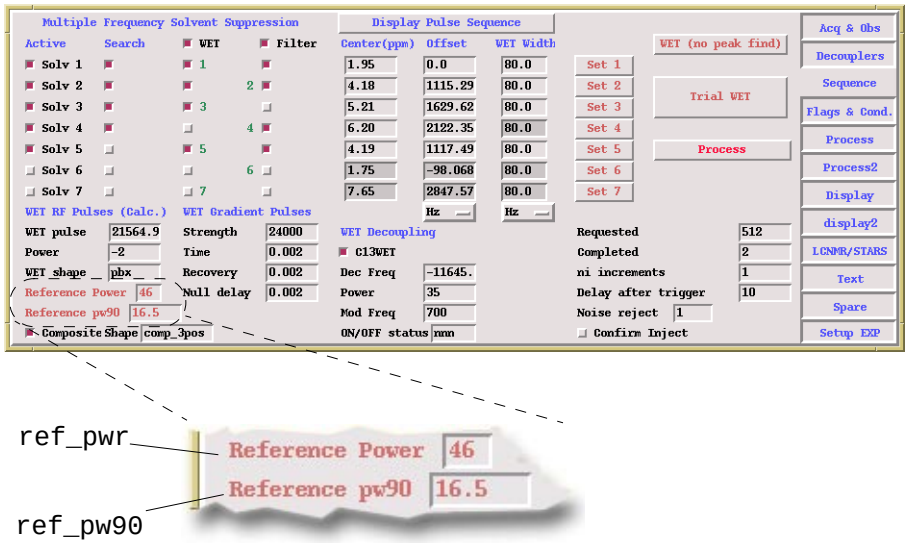


Figure 114. lc1d Sequence Pane

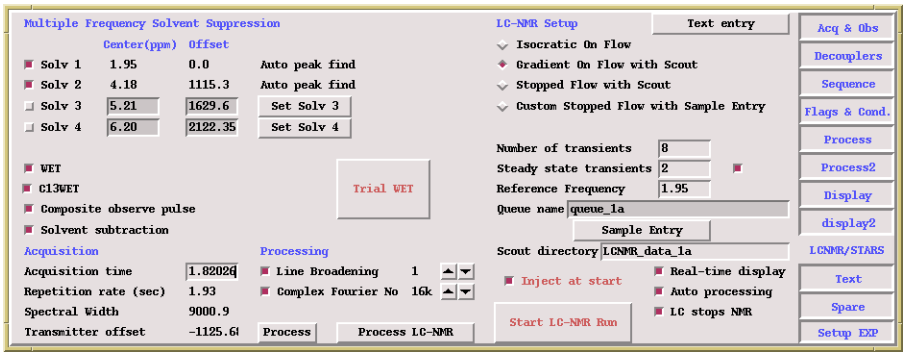


Figure 115. LC-NMR Pane

does not have to be selected to use Solv 3, or Solv 4. A more detailed discussion on how to determine the which signal suppression option to select when 2 or more signals are to be suppressed is given in step 13.

The value of the Center and Offset for Solv 1 is determined automatically by searching over a wide band of frequencies.

- If Solv 2 is not selected, the tallest peak in the spectrum is suppressed.
- If Solv 2 is selected, the high field signal is assigned to Solv 1 (this may or may not be the tallest signal in the spectrum) and then the region of the spectrum that is to the low field side of Solv 1 is searched for the tallest signal in that region.

Center and Offset are determined automatically and displayed. These values are non changeable on this panel. These values can, however, be entered in the corresponding Sequence pane.

For Solv 3, or Solv 4 you must supply either a frequency for Center (in ppm) or a value for the offset from the carrier for Offset (in Hz). These values can be entered manually, or they can be extracted from a spectrum by placing a cursor at the

appropriate location in the displayed spectrum and pushing the appropriate Set Solv button. When either Solv 3, or Solv 4 or both are selected, a narrow range of frequencies, about the set frequency is searched. The frequency range of the search is discussed in step 13.

Three additional NMR signals can be suppressed for a total of 7. The Sequence pane provides control of the suppression of these additional signals - see step 13.

10. **Select** the **C13WET** box to suppress the ^{13}C satellites of the solvent resonances.

    This assumes the values for Dec Freq, Power, and Mod Freq (located in the Sequence pane) have been properly set. The ON/OFF status is typically nnn, although nny may be useful if the size of the residual 13C satellites are a problem.

11. **Click** on the **Acq & Obs** tab to open the Acq & Obs pane, see Figure 116.

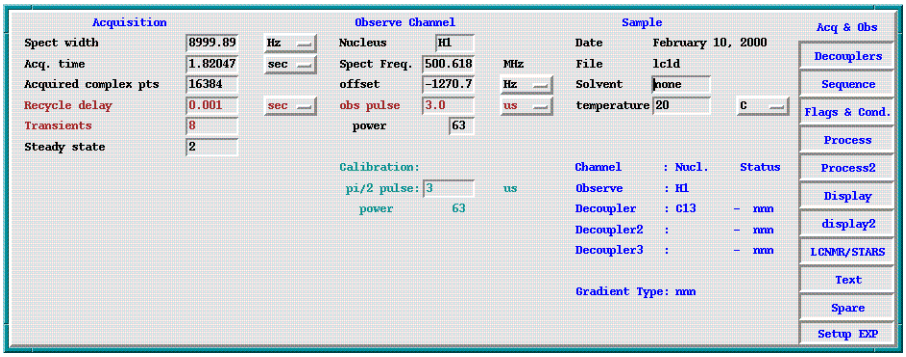


Figure 116. lc1d Acq & Obs Pane

12. The **obs pulse** and **power** should be set to value that produce `pw90` that is as short as practical. In general, the shorter the better, although 3.0 or 4.4 microsecond `pw90`s are particularly useful. see step 14.

13. Multiple Frequency Solvent Suppression Options.

    a. **Click** on the **Sequence tab** to open the **Sequence pane** for suppression of more than four signals.Select up to 7 signals using the LCNMR/STARS pane.

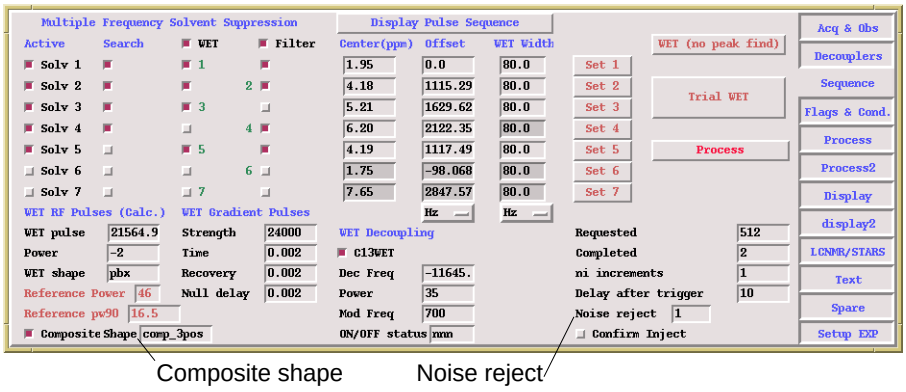


Figure 117. lc1d Sequence Pane and Frequency Suppression Options

One (must be Solv 1) or two (must be Solv 1and Solv 2) frequencies can be searched for automatically. Five additional frequencies can be searched for over a narrow range based on the value of the center frequency. In the

Sequence pane, up to 7 signals can selected. Solv 1 must be active and search selected if two or more signals are to be suppressed.

Both Solv 1 and Solv 2 (see step 9 for description of Solv 1 and Solv 2 frequency search) use algorithms for finding the signal frequency that are different from the algorithm used by Solv 3 through Solv 7 to locate the signal frequency. If the search option is active for any one of four signals, Solv 3 through Solv 7, the search is restricted to an approximate range of + or - 0.2 ppm about the value displayed in the Sequence pane for the Center frequency. The Center frequency can be either entered manually or set by placing the cursor over the signal and pressing any one of the SET buttons. The value of Offset is calculated automatically.

b. Specify **acquisition and processing options** for each signal. Each **Solv** selection, see Figure 117, has four choices:

– Search for a signal.

– Suppress a signal using **WET.**

– Apply post acquisition signal suppression **filtration.**

– Specify the **Wet Width** of the signal suppression.

Each choice can be applied individually or in any combination. The choices are only active if the Active button for a given Solv is RED, see Figure 117. Currently the software limits post acquisition filtration to a maximum of 4 signals (more then four then filter buttons to may be set to active in the tcl pane). If five or more filter buttons are active, only the first four active Solv with filtration selected are active.

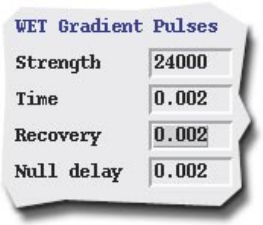
Wet Width is typically set to 80 Hz (for each solvent frequency; this corresponds to about a 20 msec pulse). You can adjust these entries to larger or smaller values to generate a larger or smaller bandwidths of suppression for each signal (as desired). This value of Width determines the duration of the WET pulse, which influences the power of the WET pulse (both parameters being displayed in the Sequence pane).

Pressing the **Wet (no peak find)** button executes scan using the parameters as they appear in the Sequence window. WET suppression and or post acquisition Filtering is only applied to those signals that are Active and have either or both of these options selected.

Pressing the **Trial WET** button executes a search for each active signal with search enabled and apply all other options that are enabled for each active solvent.

14. **Select Composite observe pulse** in the **LCNMR/STARS** pane (Figure 115) if you can achieve a `pw90` of 3.0 or 4.4 µs. This produces better looking data. Comp shape in the Sequence pane (Figure 117) must also be properly set (for pw=3.0 use comp_3g and for pw=4.4 use comp_44g).

15. In the **Sequence** pane, the parameters under WET Gradient Pulses are typically set as follows:



Label	*Value*	*Parameter Equivalent*
Strength	**2400**	`gzlvw`
Time	**0.002**	`gtw`
Recovery	**0.002**	`gswet`
Null delay	**0.001**	`dz`

Because the solvent resonances are often left upside down in relation to the peaks of interest, the Null delay can optimized to a value between 0 and 0.005 to catch the signals as they pass through zero (in analogy to the `WEFT` experiment).

16. Set **Noise reject** to **3** in the **Sequence** pane, see Figure 117.

    The Noise reject parameter affects the determination of the frequencies for solvents 2 through 7, but not solvent 1. Set Noise reject to a higher or lower number as appropriate:

    - To maximize the reliability of peak-finding for large solvent resonances, set Noise reject to a larger number (5 to 10).
    - To detect smaller resonances, set Noise reject to a smaller number (1 to 3).

    Noise reject does not accept values of less than 1.

    - If Noise reject is set too small, the Scout Scan™ incorrectly detects an incorrect frequency to suppress (e.g., a noise spike or a tall sample resonance).
    - If Noise reject is set too large, the Scout Scan™ does not detect the peak you intend to suppress (in which case Scout Scan™ sets the frequency to the last value used, which might not be accurate for the current sample), although this is a useful way to suppress a fixed frequency or a very tiny signal.

17. Click on the **Trial WET** button in the **LC-NMR** pane (Figure 115) to perform further optimizations. This starts the Scout Scan™ process. Two spectra are produced:

    - The first spectrum is a one-transient no-solvent-suppression 1D spectrum (full spectral width). The NMR software spends a few seconds analyzing this data, creating the appropriate shapes, and setting up parameters.
    - The second spectrum, a signal-averaged data set, is acquired using the parameter optimization that was done after the initial acquisition completed. The data is automatically Fourier transformed and displayed as the second spectrum.

Troubleshooting Solvent Suppression

If the suppression does not look as good as it should, the cause may be one of the following:

- PFG gradients are not properly connected, turned on, or configured (check the values of `gradtype` and `pfgon`).
- Probe is not well rinsed out.
- Shims are not optimized. Note that the shims can change significantly between acetonitrile and methanol solvent mixtures.
- Parameters `ref_pwr` and `ref_pw90` are incorrect for the probe currently in use.

Evaluating Solvent Mixture Equilibration

The NMR chemical shifts of the solvent signals change as a function of mobile phase composition. You should always allow the pumped LC solvent mixture to equilibrate before starting an NMR run. One way to evaluate this is by doing the following:

1. In the **LC-NMR** pane, press the **Trial WET** button.
2. After Trial WET finishes, check the value of transmitter offset as well as the **Offset** values for any selected **Solv 2**, **Solv 3**, and **Solv 4** entries.

3. Repeat 1 and 2 until these values remain constant. The system is equilibrated when these numbers do not change after you run Trial Wet.

   The offsets for Solv 1 and Solv 2 are usually determined by the Scout Scan. They are not directly changeable by the user in the LC-NMR pane, but manual entry is possible in the Sequence pane.

Solvent Suppression: Background Information

The Trial WET button in the LC-NMR pane is not affected by the Inject at start parameter. The only time an injection can be accomplished from the NMR is when the Start LC-NMR Run button is clicked (and then only if the Inject at Start mode is selected).

For LC-NMR, the LC-NMR pane and the Sequence pane are of the most interest:

- Virtually all routine LC-NMR operations are available from the LC-NMR pane.
- The Sequence pane changes to reflect the needs of different experiments, for example, if `wettocsy.c` or `wethmqc.c` is selected.

WET Experiments

Using a modified form of the WET (Water Eliminated through Transverse gradients) experiments, the peaks of (dis)interest are excited with a selective 90˚ pulse, using a WET waveform that has been convoluted with one or more frequencies (SLP). This pulse is then followed with a gradient to dephase the undesired signals, and the process is repeated multiple times. The WET shape has been found to be a suitable choice for both on-flow and stop-flow LC-NMR applications.

WET Shapes

The WET shape used (set to `pbx` in the Sequence pane) is calculated on-the-fly by the Pbox software package, which is now resident within VNMR. This shape is recalculated many times during an LC-NMR experiment. For this reason, the single shape name `pbx` is used by the pulse sequence although the shape is recalculated for each use.

While a number of basic shapes can be used to apply selective pulses to solvent lines, there are always tradeoffs. Longer pulses are more selective, but require more time (which is of the essence, particularly in flowing systems). WET (based on SEDUCE-1) is a shape taken from the literature that has been found to be very effective in pulsed field gradient solvent suppression experiments.

One of the two shaped pulses `comp_3g.RF` or `comp_44g.RF` is used when the Composite observe pulse box in the LC-NMR pane is selected. The user can define which of these is used in the Comp shape field in the Sequence pane.

Related Parameters

Solvent suppression is achieved not only by the pulse sequence, but also (in part) by software processing. Software processing is most frequently accomplished by using `wftlc` after the parameters `ssfilter` and `ssntaps` (and perhaps `ssorder`) have been set to reasonable values (typically, `ssfilter` is set to about `80`, `ssntaps` is set to about `251`, and `ssorder` is not used).

Another parameter of interest is `sslsfrq`. This parameter shifts the location of the filter notch (in Hz from `tof`). Often, the quality of solvent suppression can be improved somewhat by setting `sslsfrq` to a small positive number (e.g., `sslsfrq=3`). This parameter can also be arrayed to produce multiple filter notches within the same spectrum.

Selection of which signal to which the notch filter is applied is made in the **Sequence** pane. Any four of the available seven signals may be selected. Note that the filter is applied only to the first four signals if more then four are selected.

13.3 Maximizing LC Sensitivity

Several factors affect the sensitivity of LC-NMR experiments. Generally, the concentration of analyte (eluted component) in the probe and the length of time the analyte is in the probe can be easily controlled for maximum sensitivity.

On-flow experiments generally have relatively short residence times in the probe but provide the best overview of your sample. Stop-flow experiments allow using as much time as required to acquire a spectra but do not provide continuous spectra of the sample as it elutes. In both types of experiments, inject as much sample as possible.

A consideration when conducting either on-flow or stop-flow experiments is band broadening. Once a sample is injected into the HPLC, the sample mixes with solvent and the sample plug begins to increase in volume. As the sample passes through the column, it is separated into its components. As each component (analyte) elutes from the column, it mixes with the solvent and continue to grow in volume thus decreasing its concentration.

Minimizing the volume of extra tubing connecting the HPLC system and NMR helps to maximize the concentration of analyte reaching the probe. If possible, increasing the concentration of the sample and subsequently using a proportionally smaller sample loop on the injector (thus injecting the same amount of sample) increases the concentration reaching the probe as well.

If the sample is amenable to a gradient separation, you may also be able to inject the sample while the weak solvent is flowing through the system, thus causing the components of the sample to concentrate at the column head. An appropriately designed gradient may then provide sharper peaks than you would otherwise get.

13.4 On-Flow LC-NMR Experiments

During on-flow LC runs, the NMR system takes spectra “on-the-fly” as the chromatogram elutes from the HPLC system, resulting in a data set that samples the chromatogram at regular intervals. On-flow LC runs require nothing more than starting the HPLC system after the NMR system begins acquiring data.

On-flow experiments use the NMR spectrometer to monitor the effluent from the HPLC at regular intervals with out stopping the HPLC pump. This technique is useful if you have limited sample and have intense, easily observed components in the sample. During on-flow runs, the Cascade program coordinates the start of the HPLC with the beginning of acquisitions by the NMR system.

On-flow LC runs can be run as *isocratic* or *gradient*.

- *Isocratic* runs require the solvent ratios to remain the same throughout the run (see “Acquiring Isocratic On-Flow LC-NMR Data,” page 411).
- *Gradient* runs change the solvent ratios throughout the run (see “Acquiring Gradient On-Flow LC-NMR Data,” page 413).

Number of Transients in On-Flow Experiments

The number of transients per spectra is usually between 4 and 36, with an acquisition time of 1 to 2 seconds per transient. For a typical chromatogram eluting from a 4 mm i.d. column at 1 mL/min, a safe starting point is between 8 and 16 transients taken for each spectrum, using a 1 second acquisition time and either no or very little recovery delay (`d1`) set. Use more transients if you are using lower flow rates.

The width of the peaks being observed is the key to deciding how many transients for each spectrum (or increment) are required. The broader the peak, the more transients you can use. If the time required to acquire the spectrum for a given HPLC peak is too long, you lose sensitivity by including baseline into your spectrum. If the time is too short, you take several slices through the peak, resulting in several spectra for each peak with each spectra having a low signal-to-noise.

A good rule of thumb is to choose the number of transients on the NMR system so that the time required to complete those transients is approximately equal to the width of the narrowest HPLC peak at one-half its height.

Sensitivity in On-Flow Experiments

One strategy for maximizing sensitivity of on-flow experiments is to inject as much of your sample as possible. The trade-offs between injecting very large and moderate amounts of material are signal strength versus resolution.

Large injections yield good signal strength but may severely overload the column. Column overload can cause a loss of resolution between peaks that are close together. If your chromatogram contains well-separated peaks, you should use as much sample as is practical and convenient for your injections.

By slowing the flowrate, you increase the residence time of the analyte in the probe, allowing you to signal average for a greater period of time. For on-flow experiments, you should choose a number of transients and acquisition time about equal to a peak's width at half height (note that the width at half height for a peak is listed by the Varian Star Chromatography workstation in its reports).

Scout Scan Solvent Suppression

The method that adjusts solvent suppression parameters on-the-fly is called Scout Scan. Scout Scan takes a quick one-pulse *scout* spectrum, including the solvent peaks, uses that spectrum to determine solvent suppression parameters, and then takes a *real* spectrum (usually involving a larger number of transients, e.g., 16), alternating back and forth between these spectra as the run proceeds.

A small amount of the time during the run is used up by the Scout Scan, but against this disadvantage are weighed two advantages:

- Removal of the need for the blank run, which requires time and solvent.
- Improved solvent suppression, because changing conditions such as temperature, column packing, etc. frequently mean that gradients are not perfectly reproducible.

Acquiring Isocratic On-Flow LC-NMR Data

During isocratic LC runs, the solvent ratios remain constant throughout the run. This means that the solvent-suppression frequencies determined at the beginning of the run can remain constant throughout the entire experiment.

1. Set up the LC system for an isocratic run by setting up LC conditions, detector wavelength, and runtime on the LC hardware.
2. Select **Inhibit Apex Detection** in the Cascade program. This sets the LC system to ignore peaks.
3. Set up solvent suppression parameters in VNMR, as described in "Setting Up Solvent Suppression," page 404.
4. Load the sample into the loop:
   a. Load the sample into the syringe.
   b. Use the syringe to inject the sample into the injector valve.
5. Allow the mobile phase in the NMR flow cell system to equilibrate.
   a. Click the **Trial WET** button in the **LC-NMR** pane.
   b. After Trial WET finishes, check the value of transmitter offset (`tof`) as well as the **Offset** values for any selected **Solv 2** through **Solv 4** entries. If more then four solvent resonances were suppressed, click the **Sequence** pane and check the values `tof` and **Offset** for **Solv 1** through **Solv 7**.
   c. Repeat a and b until the Offset values remain constant. The system is equilibrated when these numbers do not change after you run Trial WET.
6. Select **Isocratic On Flow** in the **LC-NMR** pane.

   This selection is located under LC-NMR Setup in the LC-NMR pane.
7. Select **Inject at Start** to inject the sample at the start of the LC run.
8. Select **Real-time display** to display data as it is acquired.
9. Select **Auto processing** if you want the acquired data to be process automatically after the LC run is finished.
10. Select **LC Stops NMR** in the LC-NMR pane.

    The NMR software runs until it receives a stop signal from the LC; then, NMR acquisition is aborted and automatic NMR data processing and display o LC and NMR data are initiated.
11. Click the **Text entry** button to update the experiment description file.
12. Click the **Start LC-NMR Run** button to begin the isocratic run.

    The settings for the Inject at start, Real-time display, and Auto processing (at the end of the experiment) are used, as well as the values of Number of transients and Steady state transients.

    Using a `go`, `ga`, or `au` at this point to start the acquisition, does not produce the desired affect. The Start LC-NMR Run button performs some additional background calculations with the Increments number, and these calculations are required for proper operation.

To Abort the Experiment

If you abort the experiment before it completes, the data must be processed manually (see "Data Processing Methods," page 429).

1. Enter **`aa`** in the VNMR input window.
2. Click **Stop Run** in the Cascade program.
3. Reset the LC pump and detector.

To Process the Data

If you did not select Auto processing, you can process the data after the LC run is finished as follows

- Click the **Process LC-NMR** button in the LC-NMR pane.

  – or –

- Enter **`wftlc`** in the VNMR input window.

Refer to "Data Processing Methods," page 429 for more information.

Acquiring Gradient On-Flow LC-NMR Data

Because the solvent ratios change throughout a (solvent) gradient LC-NMR experiment, and the resonance frequencies of each solvent are usually influenced by the solvent ratios, the NMR system must compensate for these changing solvent positions. This solvent information is provided to the NMR system in real-time by using the Scout Scan method.

1. Set up the LC system for a gradient run by setting up LC conditions, detector wavelength, and runtime on the LC hardware.
2. Select **Inhibit Apex Detection** in the Cascade program so that the LC system ignores peaks.
3. Set up solvent suppression parameters in VNMR, as described in "Setting Up Solvent Suppression," page 404.
4. Load the sample into the loop:
   a. Load the sample into the syringe.
   b. Use the syringe to inject the sample into port 1 on the inject valve.
5. Allow the mobile phase in the NMR flow cell system to equilibrate:
   a. Click the **Trial WET** button in the **LC-NMR** pane.
   b. After Trial WET finishes, check the value of transmitter offset (`tof`) as well as the **Offset** values for any selected **Solv 2** through **Solv 4** entries. If more then four solvent resonances were suppressed, click the **Sequence** pane and check the values `tof` and **Offset** for **Solv 1** through **Solv 7**.
   c. Repeat a and b until the Offset values remain constant. The system is equilibrated when these numbers do not change after you run Trial WET.
6. Select **Gradient On Flow with Scout** in the **LC-NMR** pane.

   This selection is located under LC-NMR Setup in the LC-NMR pane.
7. Enter a directory name in the **Scout directory** field.

   A directory name is required for solvent-gradient operation. The data files are acquired individually and are initially stored in a new directory called `Scout Directory`. The data files themselves are assigned file names of `1.fid`, `2.fid`, and so on within the parent `Scout` directory.
8. Select **LC Stops NMR** in the LC-NMR pane.

   The NMR software runs until it receives a stop signal from the LC; then, NMR acquisition is aborted and automatic NMR data processing and display o LC and NMR data are initiated.
9. Select **Inject at Start** to inject the sample at the start of the LC run.

10. Select **Real-time display** to display data as it is acquired.
11. Select **Auto processing** if you want the acquired data to be process automatically after the LC run is finished.
12. Click the **Text entry** button to update the experiment description file.
13. Click on the **Start LC-NMR Run** button to begin the solvent-gradient run.

    The value of Steady state transients is not used in this experiment. If the Confirm Inject option on the Sequence pane is selected, you are asked to confirm that you want to inject.

    Using a `go`, `ga`, or `au` at this point to start the acquisition does not produce the desired results. The Start LC-NMR Run button performs some additional background calculations, which are required for proper operation.

To Abort the Experiment

If you abort the experiment before it completes, the data must be processed manually (see "Data Processing Methods," page 429).

1. Enter **`aa`** in the VNMR input window.
2. Click **Stop Run** in the Cascade program.
3. Reset the LC pump and detector.

To Process the Data

If you did not select Auto processing, you can process the data after the LC run is finished as follows:

- Click the **Process LC-NMR** button in the LC-NMR pane.

Refer to "Data Processing Methods," page 429 for more information.

Gradient LC-NMR data is processed using the `add/sub` buffer (experiment 5). Data previously left in experiment 5 is flushed. You can start a solvent gradient LC-NMR experiment in experiment 5, but you will be unable to process the data until processing is initiated in an experiment other than experiment 5.

The information stored in text is replaced with the words "`new experiment`" during this processing.

13.5 Stop-Flow LC-NMR Experiments

Stop-flow experiments allow you to stop analytes (eluted components) in the NMR probe. Once an analyte is parked in the probe, you can signal-average for an extended time, thus increasing your sensitivity to peaks that you would otherwise not easily observe in on-flow experiments. Stop-flow experiments are required for those analytes that can not be observed on-flow and for NMR experiments that require more than a few seconds to complete.

You can do stop-flow experiments in a *stop-flow* mode or *loop-collection/loop-flushing* mode:

- *Stop-flow mode* – Involves stopping during the run to perform the experiments.
- *Loop collection/ loop-flushing mode* – Involves a series of *loops* (i.e., *fractions*) that are collected during the course of an LC run and subsequently back-flushed into the LC-NMR probe.

In both modes, one or more experiments can be performed on each LC peak.

Acquisition setup for stop-flow or loop-flushing runs provides two ways to run the experiments:

- *Stop-Flow with Scout* – Assumes you want to do one experiment on each sample (typically a 1D ^{1}H spectrum) and all with the same number of transients (assuming the experiment is run fully automated).
- *Custom Stop-Flow with Enter* – Assumes you want the ability to run a number of experiments on each sample, and the selection for each sample in the queue might be different. This mode uses the `enter` program that is modified for LC-NMR to input information about each experiment to be performed (see "Acquiring Stop-Flow LC-NMR Data Using Enter," page 418). One difference is that the `Sample#` does not refer to a sample in a sample changer, but rather to the peak number in the stop-flow run, or to the loop number in the loop-flushing mode. In this case, you currently need to use sequential (and continuous) numbers, although the defined experiment can be made quite short (`nt=1`; however, the program acquires data on each `Sample #`).

  The Save And Exit button in `enter` is used to write the experiment queue out to a file (e.g., `lcqueue`). We are now acting like the queue of sample peaks (or loops) just represents another sample changer, only we are using special macros to ensure that the parameters involved in running the Scout Scan and then setting up solvent suppression are properly handled. Global parameters `autodir` and `autoname` as well as the local parameter `savefile`, are used to keep track of the run; all these parameters are created automatically. In addition to the normal automation parameters, in LC runs, the parameters `ntrig` and `dtrig` are also created to specify the number of triggers to wait for and the time to delay after each trigger (to allow adjustment for the time it takes for conditions to settle after the peak arrives at the probe; this delay can also be adjusted at the LC end).

How Stop-Flow LC-NMR Works

To perform stop-flow experiments, you need an HPLC detector to provide an analog signal to the Cascade program through the ADC board installed in the LC STAR workstation. When the signal from the detector exceeds the threshold criteria you have established, the Cascade program begins searching for an apex. Once found, the system begins counting the delay time, which is the time it takes the peak to go from the LC to the probe. When the delay time has elapsed, the stop-flow valve is activated, stopping the analyte in the probe, and a signal is sent to the NMR spectrometer to begin acquiring spectra. The signal to the NMR is read by the `ntrig` parameter of the NMR system. In order to read the Cascade program's start signal, `ntrig` must be set to one (`ntrig=1`). Alternatively, you may elect to set up the NMR system to ignore the signal and acquire the spectrum yourself.

The Cascade program responds to peak apexes and to flat lines caused by peaks that exceed the dynamic range of the HPLC detector. Peaks that exceed the dynamic range and consequently cause a flat line detection mode are not stopped in the probe as reliably as peaks that exhibit a sharp apex. This is a result of the uncertainty in where the apex occurs during the time the HPLC detector is saturated. You can avoid this situation by monitoring your intense analytes at wavelengths that are removed from the absorption maxima of the compound. Generally, this means going to longer wavelengths, where the absorption is substantially less and where end absorption effects of either the solvent or the sample components do not interfere.

Sensitivity in Stop-Flow Experiments

While the residence time of the analyte in the probe is not an issue in stop-flow experiments, you should still maximize the concentration of your analyte in the probe to allow the best spectra in the least time. Realize that, in addition to the issue of the residence time in the probe, all the other factors that influence sensitivity in an on-flow run are the same as in a stop-flow run (i.e., good chromatography, minimal mixing, and so on) and must be optimized.

Setting The Threshold and Slice Width Values

The threshold value must be set for automatic or time-slice stop-flow runs and the slice width value is used for time-slice runs. The threshold value serves as a kind of filter, allowing the Cascade program to ignore peaks that are below the threshold. After a threshold is reached, the Cascade program begins searching for the peak apex (assuming the Inhibit Apex Detection box is unchecked).

The slice width value is the number of seconds the pump should run between spectra in a time-slice experiment. For best resolution, set the slice width so that 4 or 5 spectra are taken across your peak of interest.

The threshold value is based on the sample and can be determined as follows:

1. Select **Inhibit Apex Detection** in the Cascade program so that the LC system ignores peaks.

   You can also select On Flow in the Cascade System Configuration window.

2. Set **Full Scale** to a value appropriate to your sample. If you expect large peaks, start by setting the **Full Scale** value to the maximum.

3. Set the **Display Offset** to a value appropriate for your sample.

   This setting is useful if you wish to see negative excursions.

4. Load the sample into the loop:

   a. Load the sample into the syringe.

   b. Use the syringe to inject the sample into the injector valve.

5. Click the **Start** button in the Cascade program.

6. Watch for the peak of interest in the chromatogram trace in the Cascade window.

   - When the peak of interest appears, determine a threshold value that is below the peak apex. The threshold value should be above any smaller peaks you consider to be noise or nonessential.
   - If you plan to do a time slice, determine a slice width value that gives you 4 or 5 slices across the peak.

7. Allow the system to finish the run. The run should be long enough to push the sample all the way through the probe.

8. Open the Cascade **System Configuration** window and enter the threshold value in the **Default Threshold** field.

Acquiring Stop-Flow LC-NMR Data Using Scout

Use this procedure if you want to run the same experiment on each *sample* (e.g., a 1000-scan 1D spectrum). Each *sample* flushed into the probe triggers a Scout Scan (Trial WET) routine, followed by a signal-averaged 1D NMR run.

1. Open the Cascade **System Configuration** window. Click on **On-Line/Stop Flow in**.
2. Set **Default Hold** to a value longer than the duration of the longest NMR experiment you are performing. The value, in minutes, is how long the Cascade program waits for the NMR experiment before restarting the pump.

   If you want the LC system to wait for the NMR to finish before the pump is restarted, select **Wait for NMR Complete**. The Cascade program ignores the Default Hold and waits for a signal from the NMR system.
3. Check the **Delay** value and make sure it is appropriate for your flow rate and transfer tube length. This value is determined using the procedure in "Delay Time Calibration," page 431.
4. Make sure the **Threshold** value is correct for your sample, as determined in the previous procedure.
5. In the Cascade program window, make sure **Inhibit Apex Detection** is **not selected**.
6. Load the sample into the loop:

   a. Load the sample into the syringe.

   b. Use the syringe to inject the sample into the injector loop.

   The sample is held in the loop until the NMR requests a sample from the loop by sending a signal to the PC, or until the pump or Cascade program are started.
7. Press the **Pump** button on the LC pump.

   Solvents begin pumping through the system. Allow the system to pump until the solvents are transferred into the probe flow cell.
8. Set up solvent suppression parameters in VNMR as described in "Setting Up Solvent Suppression," page 404.
9. In the LC-NMR pane in VNMR, select **Stopped Flow with Scout**.
10. Enter values in the **Number of transients** and **Steady state transients** fields.
11. Enter a directory name in the **Scout directory** field.

    This is required for solvent-gradient operation. The data files are initially acquired one-at-a-time and are stored in the directory called entered in Scout directory. They are stored in the format `0101.fid`, `0102.fid`, and so on, as specified by the `autoname` parameter as reassigned within the LC-NMR macros. Each *peak* produces two spectra: the `0#01` spectrum is the (saved) Scout Scan data, while the `0#02` spectrum is the solvent-suppressed (desired) data set.

    This process starts the automation run with an already predefined queue for 96 samples (which is stored in either `/vnmr/asm/autoscout_enterq` or in `~/vnmrsys/asm/autoscout_enterq`). The sample information is used to automatically create the output file of the `enter` program, which is located in `enterQ`.

    To check the run, enter **`status`** in the VNMR input window.
12. Select **Inject at Start** to inject the sample at the start of the LC run.

The Real-time display and Auto processing selections have no meaning in this experiment. The acquisition takes place in background within VNMR, which allows you complete freedom to process another data set in any desired experiment location.

13. Click the **Text entry** button to update the experiment description file.
14. Click the **Start LC-NMR Run** button in the LC-NMR pane to begin the stop-flow run.

    Using a `go`, `ga`, or `au` at this point to start the acquisition does not produce the desired results. The Start LC-NMR Run button performs some additional background calculations, which are required for proper operation.

    If you are satisfied with the acquired data before the set number of acquisitions are reached, enter `halt` in VNMR to move on to the next peak (or loop).

    If you enter `aa` instead of `halt`, the program saves the data and moves on, but `wexp` functions do not take place. You then need to click the Abort Hold button in the Cascade program to cause the LC system to progress to the next peak (or loop).
15. To review the data, enter **`status`** in the VNMR input window.

    The files, which are stored in the Scout directory, can be read back into the current experiment and reprocessed automatically by selecting the Retrieve Data button within the Status window. At that point, processing parameters can be altered and the data set reprocessed (using selecting the Process button in the LC-NMR pane or by entering `wft` in the VNMR input window.

    Two files are saved for each peak or fraction. The first file (`0#01.fid`) is produced by the Scout Scan run (`nt=1`) while the second file (`0#02.fid`) is the proper signal-averaged data.

Acquiring Stop-Flow LC-NMR Data Using Enter

Use the following procedure if you want to run different experiments on each *sample*. This sets up a VNMR automation-mode process that runs in background (like Stopped Flow with Scout), but which can be customized through the use of the `enter` program.

Each *sample* flushed into the probe triggers a Trial WET routine, followed by a signal-averaged 1D NMR run. The NMR parameters listed in the current experiment are used by the `autoscout` macro to acquire the data unless otherwise modified with the `enter` program.

1. In the **Cascade program**, select **Configuration** from the **Setup** pull-down menu.
2. Select **On-Line/Stop Flow**.
3. Set **Default Hold** to a value longer than the duration of the longest NMR experiment you are performing. The value, in minutes, is how long the Cascade program waits for the NMR experiment before restarting the pump.

   If you want the LC system to wait for the NMR to finish before the pump is restarted, select **Wait for NMR Complete**. The Cascade program ignores the Default Hold and waits for a signal from the NMR system.
4. Check the **Delay** value and make sure it is appropriate for your flow rate and transfer tube length. The value is determined using the procedure in “Delay Time Calibration,” page 431.
5. Make sure the **Threshold** value is correct for your sample (refer to “Setting The Threshold and Slice Width Values,” page 416)

6. Open the Cascade window and make sure **Inhibit Apex Detection** is **not selected**.
7. In the VNMR Configuration program, make sure that the **`Sample Changer`** is set to **`LC-NMR`**. You may have to log in as `vnmr1` to do this.
8. Set up solvent suppression parameters as described in "Setting Up Solvent Suppression," page 404.
9. Load the sample into the loop:
   a. Load the sample into the syringe.
   b. Use the syringe to inject the sample into the injector valve.

   The sample is held in the loop until the NMR signals a sample injection or until the pump or Cascade program are started.
10. Press the **Pump** button on the LC pump.

    Solvents begin pumping through the system. Allow the system to pump until the solvents are transferred into the probe flow cell.
11. In VNMR, click on the **LCNMR** tab to open the LC-NMR pane.
12. Select **Custom Stopped Flow with Enter**.
13. Set reasonable values for **Number of transients** and **Steady state transients**.
14. Enter a file name for **Queue name** and a directory for **Scout directory**.

    The file name you enter for Queue name stores the information set by the `enter` program.
15. Click the **Sample Entry** button.

    The Sample entry Form window appears, as shown in Figure 118.

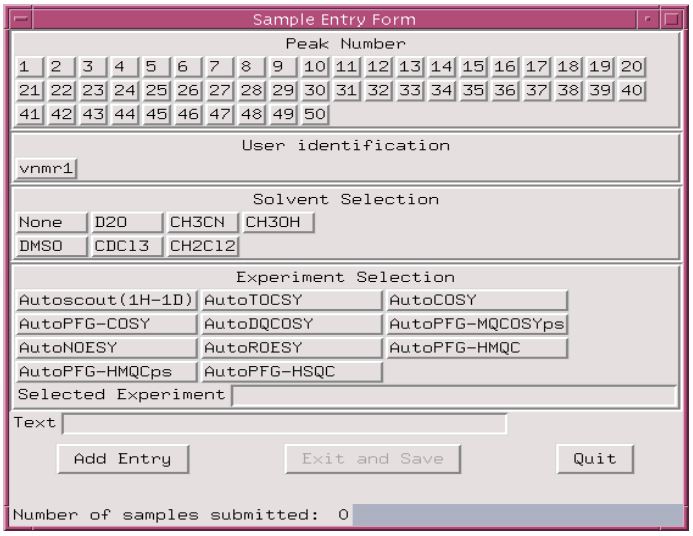

Sample Entry Form
Peak Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50
User identification
vnmr1
Solvent Selection
None D2O CH3CN CH3OH
DMSO CDCl3 CH2Cl2
Experiment Selection
Autoscout(1H-1D) AutoTOCSY AutoCOSY
AutoPFG-COSY AutoDQCOSY AutoPFG-MQCOSYps
AutoNOESY AutoROESY AutoPFG-HMQC
AutoPFG-HMQCps AutoPFG-HSQC
Selected Experiment
Text
Add Entry
Exit and Save
Quit
Number of samples submitted: 0

Figure 118. LC-NMR Sample Entry Form Window

16. Fill in the Sample Entry Form window.
    - **Peak Number** – Select one or more peaks sequentially on which to run experiments.
    - **User Identification** – Select the user.

- **Solvent Selection** – Select the solvent for the chosen peak(s). Typically, **None** is selected.
- **Experiment Selection** – Select an experiment from the list. It should match the solvent in the original parameter set.
- **Text** – Enter information about the experiment, if desired.
- When finished with this peak (or peaks), click **Add Entry**.

When you are done adding entries, click **Exit and Save**.

The macro `autoscout` is used here and uses the NMR parameters set in the current foreground experiment as its starting parameter set. The number of transients and the number of steady-state scans can be redefined by the experiment selected in the Sample Entry Form window (as opposed to what was selected in the LC-NMR pane). This happens if `/vnmr/asm/experiments.conf` has the `xradiocustom` line uncommented, and you submit an experiment like `autoscout('nt=32')`.

Many of the other parameters in the LC-NMR pane (Real-time display, Auto processing, Increments, and Scout directory) have no effect in this mode of operation. The parameter Inject at start may or may not have any effect, depending upon what experiments are setup through the enter queue.

17. Click the **Start LC-NMR Run** button.

    Note that two files are created for each peak or fraction. The first files (`0#01.fid`) arise from the Scout Scan run (`nt=1`) while the second files (`0#02.fid`) are the proper signal-averaged data.

    VNMR signals the Cascade program to begin.

    The Cascade program waits for a peak that exceeds the set threshold. After a peak reaches the threshold, the Cascade program begins searching for the peak apex. When the peak apex is detected, the delay counter begins to count while the pump continues, allowing the peak to be transferred into the flow cell of the probe. When the transfer time is counted, the pump is stopped and the NMR system is signaled to begin the acquisition.

    The pump begins again when either the hold time expires or the NMR system signals that it is finished with the experiment.

18. If you are satisfied with the acquired data before the set number of acquisitions are reached, enter **`halt`** in VNMR to have the NMR program move on to the next peak (or loop).

    If you try to use an `aa` instead of `halt`, the program saves the data and moves on, but `wexp` functions do not take place. You then need to select the Abort Hold button in the LC-NMR Stop Flow program to cause the LC system to progress to the next peak (or loop).

    After the last experiment is collected, the spectrometer does not activate LC. So that the pump is stopped for overnight experiments, the LC is left in the hold position. To end the experiment, click Abort Stop Flow in Cascade. The LC finishes the run and sends all data to the NMR. *If Stop LC-NMR Run is pressed, data does not transfer properly.*

13.6 Time-Slice Stop-Flow Experiments

Another way to run a stop-flow experiment is the time-slice experiment. This experiment takes multiple spectra (or time-sliced spectra) over the chromatographic peak(s) being studied. The time-slice experiment is useful in analyzing an HPLC peak that is suspected of containing more than one component. The Cascade program stops the pump and turns the stop-flow valve, stopping whatever is currently in the NMR probe. A spectrum is taken and the process repeats at operator-adjustable intervals.

Creating a Time-Slice Program in Cascade

You can create a time-slice program in the Time Program window (see Figure 47) to set the start time, event, and action or end time for each event. You can set up to 16 events.

1. In the Cascade program, select **Timed Events** from the **Setup** pull-down menu.
2. Enter the start time, end time, event (in this case, Time Slice), and value.
3. Click **Save** when finished creating the time program.
4. Start your LC run as normal.

   Time slicing starts automatically at the time set in the time-slice program.

Acquiring Time-Slice Stop-Flow Data—Completely Automatic

The following procedure sets up a fully automated process that uses a VNMR automation-mode process (that runs in background) to steps through sequential *slices* (time-slice spectra) of an LC chromatogram. This is usually used to verify purity (or impurity) of a given chromatographic peak through inspection of the consistencies (or inconsistencies) of the NMR spectra. Each *sample* flushed into the probe triggers a Trial WET routine, followed by a signal-averaged 1D NMR run.

1. Press the **Start** button on the LC pump.

   Solvents begin pumping through the system. Allow the system to pump until the solvents are transferred into the probe flow cell.
2. Create a time-slice program as described in the procedure “Creating a Time-Slice Program in Cascade,” page 421.
3. Load the sample into the loop:

   a. Load the sample into the syringe.

   b. Use the syringe to inject the sample into the injector valve.

   The sample is left in the injector loop until the NMR signals for an injection by sending a signal to the PC, or until the pump or Cascade program are started.

   The Cascade program triggers the stop flow valve, stops the pump, and sends a signal to the NMR system to begin acquiring data. After the NMR system returns a signal, the pump restarts for the set number of seconds.
4. Set up solvent suppression parameters within VNMR, as described in “Setting Up Solvent Suppression,” page 404. Make sure that the blocksize parameter **bs** is set to a reasonable number (e.g., `bs=16`). Set **nt** to the desired number of transients (e.g., `nt=1000`).

   You should run a stop-flow experiment in VNMR, which provides for two types of stop-flow experiments: Stopped Flow with Scout and Custom Stopped Flow with Enter.

5. In VNMR, click the **LCNMR** tab.
6. In the LC-NMR pane, select **Stopped Flow with Scout**.
7. Set reasonable values for **Number of transients** and **Steady state transients**.
8. Enter a file name for **Queue name** and a directory for **Scout directory**.

   The file name you enter for Queue name stores the information set by the `enter` program.
9. Click the **Sample Entry** button.

   The Sample entry Form window appears, as shown in Figure 118.
10. Click the **Start LC-NMR Run** button (either with or without Inject at start selected).
11. Fill in the Sample Entry Form window.
    - **Peak Number** – Select one or more peaks sequentially on which to run experiments.
    - **User Identification** – Select the user.
    - **Solvent Selection** – Select the solvent for the chosen peak(s). Typically, **None** is selected.
    - **Experiment Selection** – Select an experiment from the list. It should match the solvent in the original parameter set.
    - **Text** – Enter information about the experiment, if desired.
    - When finished with this peak (or peaks), click **Add Entry**.

    When you are done adding entries, click **Exit and Save**.
12. Click the **Text entry** button to update the experiment description file.
13. Click the **Start LC-NMR Run** button (either with or without **Inject at start** selected).
14. To view the data being acquired, enter **`status`**, and recall the acquiring FIDs through the `status` window.

    You can only view an acquisition in progress if the blocksize parameter (`bs`) was set to a meaningfully small number (e.g., `bs` less than 128).

    If you are satisfied with the acquired data before the set number of acquisitions are reached, stop the NMR by entering **`halt`**, and then click the **Abort Hold** button to cause the LC system to progress to the next slice.

    If you are completely finished with this LC-NMR run and **LC stops NMR** was not selected, you can stop the NMR automation by entering **`autoaa`**.

    The time-slice run continues until the End Slice button is clicked, terminating the time slice mode back to the normal run mode.
15. Allow the chromatogram to continue and either stop on, or time-slice through, other peaks as desired.

Acquiring Time-Slice Stop-Flow Data—Semiautomatic

The time-slice experiment can be run semiautomatically (the VNMR process runs in foreground). Some reasons that a full-automation time-slice experiment might be unsuitable include the following:

- You want to watch the sensitivity grow with time

- You do not want to have the solvent suppression optimized with a Trial WET for each slice
- You are not sure how many transients of each slice you want
- You are not sure how many slices you need

Use the following steps to perform semiautomatic time-slice stop-flow experiment.

1. Press the **Pump** button on the LC pump.

   Solvents begin pumping through the system.

2. Download a method from the System Control page in STAR Chromatography software. Look under **file** and choose a **method** and choose **activate**.

   Allow the system to pump until the LC pressure is stable.

3. Load the sample into the loop:

   a. Load the sample into the syringe.

   b. Use the syringe to inject the sample into the injector valve.

   The sample is left in the injector loop until the pump method or the Cascade program are started.

4. In the Cascade program, select **System Configuration** from the **Setup** pull-down window to open the System Configuration window.

5. Select **Wait for NMR Complete** box.

6. Return to the Cascade program window and click the **Start Run** button.

7. When the apex of the peak of interest is detected, click the **Time Slice** button in the to stop the flow of solvent.

   Alternatively, if the peak of interest is in the probe, the flow of mobile phase can be stopped immediately by using the **Stop Now** button.

   The Cascade program switches the stop flow valve, stops the pump, and sends a signal to the NMR system to begin acquiring data.

   The pump restarts for several seconds, after one of the following occurs:

   - The NMR system returns a signal
   - Abort Stop Hold button is clicked on
   - Hold counter has expired

8. Set up solvent suppression parameters within VNMR, as described in “Setting Up Solvent Suppression,” page 404. Make sure solvent mixtures are equilibrated as described in “Evaluating Solvent Mixture Equilibration,” page 408.

9. Make sure that the blocksize parameter **`bs`** is set to a reasonable number (e.g., `bs=16`). Set **`nt`** to a unexpectedly large number (e.g., `nt=100000`).

10. Choose semiautomatic mode for one of the following:

    - Background NMR operation
    - Foreground NMR operation

    Background NMR operation –

    a. Before the Cascade program triggers the stop flow valve, click the **Stopped Flow with Scout** button on the LC-NMR pane.

    b. Enter values in the **Number of transients** and **Steady state transients** fields.

    c. Enter a directory name in the **Scout directory** field.

The directory name is required for solvent-gradient operation. The data files are initially acquired one-at-a-time and are stored in the directory called `entered` in Scout directory. They are stored in the format `0101.fid`, `0102.fid`, and so on, as specified by the `autoname` parameter as reassigned within the LC-NMR macros. Each *peak* produces two spectra: the `0#01` spectrum is the (saved) Scout-Scan data, while the `0#02` spectrum is the solvent-suppressed (desired) data set.

This process starts the automation run with an already predefined queue for 96 samples (which is stored in either `/vnmr/asm/autoscout_enterq` or in `~/vnmrsys/asm/autoscout_enterq`). This information is used to automatically create the output file of the `enter` program, which is located in `enterQ`.

d. Make sure the **Inject at Start** box is **not selected**.

The Real-time display and Auto processing fields have no meaning in this experiment. The commands `go`, `ga`, or `au` do not produce the desired results.

e. When ready, click the **Start LC-NMR Run** button.

This acquisition takes place in background within VNMR, allowing you complete freedom to process another data set in any desired experiment location. The system automatically sets up for up to 96 time-slice acquisitions.

To halt the time-slice experiment, enter **`auto_aa`**.

To view the data being acquired, enter **`status`** and recall the acquiring FIDs through the `status` window.

To view an acquisition in progress, the blocksize parameter (`bs`) must be set to a meaningfully small number (e.g., `bs` is less than 128).

If you are satisfied with the acquired data before the set number of acquisitions are reached, enter **`halt`** in VNMR to have the NMR program move on to the next slice. (Using `aa` instead of `halt` causes the program saves the data and moves on, but `wexp` functions do not occur.) You then need to click the **Abort Hold** button in the Cascade program to cause the LC system to progress to the next slice.

Foreground NMR operation—After the Cascade program triggers the stop-flow valve and the pump is stopped, manually start the NMR acquisition.

- If solvent suppression optimization is not needed, enter **`go`**.
- If solvent suppression optimization is needed, click the **Trial WET** button in the LC-NMR pane.

To view the data being acquired, push the **Process** button in the LC-NMR pane (or enter **`wft`**).

If you are satisfied with the acquired data before the set number of acquisitions are reached, save the data set manually (using **`svf`**), abort the NMR acquisition (**`aa`**), and click the **Abort Hold** button in the Cascade program to cause the LC system to progress to the next slice.

11. Allow the chromatogram to continue and either stop on, or time-slice through, other peaks as desired.

The time-slice run continues until the **End Slice** button is clicked, terminating the time-slice mode back to the normal run mode.

If you used the background mode option – When the LC experiment is finished, the NMR is undoubtedly still active and waiting for another trigger from the LC. If you

experiment is truly finished, and you have enough data, type `autoaa` in the VNMR command line to halt the NMR and empty its queue.

The background mode of operation supports data acquisition on up to 96 peaks (or loops or fractions). If the user has less than 96 peaks or fractions to analyze, the NMR continues to wait for further triggers until the NMR experiment is aborted with the `autoaa` macro. (The `autoaa` macro is equivalent to `autosa aa` and is currently required to completely abort an automation run.) Note that `autoaa` generates an (inconsequential) error in the Status window for the last experiment.

12. To review the data, enter the **`status`** command in VNMR.

    The files, which are stored in the "`Scout Directory`" directory, can be read back into the current experiment and reprocessed automatically by clicking the **Retrieve Data** button in the Status window. At that point, processing parameters can be altered and the data set reprocessed by clicking he **Process LC-NMR** button (`wftlc`), or the data set can be reprocessed without altering the processing parameters by clicking the **Process** button (`wft`).

    Note that two files are created for each slice. The first files (`0#01.fid`) arise from the Scout Scan run (`nt=1`) while the second files (`0#02.fid`) are the proper signal-averaged data.

Acquiring Time-Slice Stop-Flow Data—Manually

If you wish to perform a time-slice run manually for any reason, the time-slice experiment can be run in a manual mode. The NMR acquisition occurs in foreground in this case.

Reasons for running the time-slice experiment manually include the following:

- You want to watch the sensitivity grow with time
- You do not want to have the solvent suppression optimized with a Trial WET for each slice
- You are not sure how many transients of each slice you want
- You are not sure how many slices you need

1. Operate the LC separation entirely through the Star Chromatography software.
2. After the LC method is running, when you get close to the peaks of interest, click the **Stop Now** and **Abort Hold** buttons in the Cascade program to stop and start the flow of the mobile phase. Stop Now stops the flow and Abort Hold restarts the flow.

   You must manually synchronize these actions with the NMR.
3. Either enter **`go`** (with **`ni=1`**) or click the **Trial WET** button to start the NMR acquisition. Spectra must to be saved and processed manually.

13.7 2D LC-NMR Experiments

You can acquire 2D LC-NMR data manually or automatically by using the `enter` program. In general, if you know how to set up a standard `noesy` or `ghsqc` in VNMR, and you have learned how to set up LC-NMR solvent suppression, the WET-2D sequences are merely a concatenation of these two techniques.

- "Acquiring 2D LC-NMR Data Manually," page 426
- "Acquiring 2D LC-NMR Data Using enter Program," page 426

Acquiring 2D LC-NMR Data Manually

Because ^{1}H sensitivity is often the rate-limiting parameter in LC-NMR, more advanced 1D or 2D NMR experiments are run on stop-flow samples. After solvent suppression has been optimized for the sample in the probe, you can use a variety of macros to set up advanced experiments. The macros that start with "`wet`" (e.g., `wetdqcosy`, `wetgcosy`, `wettntocsy`, `wetnoesy`, `wetghsqc`, `wetghmqc`) are designed to use the same parameters to accomplish solvent suppression as `lc1d`, and to bring in only those parameters needed to run the advanced experiment.

In general, if you know how to set up a standard `noesy` or `ghsqc` in VNMR, and you have learned how to set up LC-NMR solvent suppression, the WET 2D sequences are merely a concatenation of these two techniques.

To manually set up a `wettntocsy` on a sample stopped in the probe, proceed as follows:.

1. Perform an on-flow or stop-flow experiment as described in one of these sections:
   - "On-Flow LC-NMR Experiments," page 410
   - "Stop-Flow LC-NMR Experiments," page 414
2. When the apex of the peak of interest is detected, click the **Stop Flow** button in the Cascade program to stop the flow of solvent.

   Alternatively, if the peak of interest is in the probe, the flow of mobile phase can be stopped immediately by clicking the Stop Now button.
3. If the sample is to be held in the probe longer than an hour, you might want to turn the deuterium lock back on for this experiment (depending upon the drift rate of your particular magnet).
4. Verify that the shims are optimized appropriately.
5. Tune the probe if needed.
6. When the system has equilibrated and stabilized, set up and optimize solvent suppression as described in "Setting Up Solvent Suppression," page 404.

   Click the **Trial WET** button in the LC-NMR pane to finalize frequency settings for the `pbox` shape.
7. Run an appropriate 1D spectrum.

   You may wish to optimize the spectral width for the 2D run; if so do it now and verify any other pulse width calibrations (if necessary).
8. Move the 1D parameters to a new experiment (`mp`), join that new experiment (`jexp#`), and enter the appropriate macro (e.g., `wettntocsy`).
9. Set up any sequence-specific parameters (e.g., `tpwr`, `pw`, `p1lvl`, `p1`, `window`, `mix`) and 2D-specific parameters (e.g., `ni`, `phase`, `nt`).
10. Enter **go** when ready.
11. Data can be saved and processed in the normal fashion for 2D NMR.

Acquiring 2D LC-NMR Data Using enter Program

To automatically set up a `wettntocsy` on a sample stopped in the probe in the `enter` queue, use the following steps.

- For each sample for which a 2D spectrum is desired, select the appropriate **Experiment** button in the **Sample Entry Form** window (e.g., `autotocsy`).

Each of these routines does the following:

- Runs a Scout Scan spectrum
- Calculates the optimized suppression parameters
- Retrieves any additional parameters required for running the 2D data set
- Starts the 2D acquisition

This procedure generates two spectra (the Scout Scan data and the 2D data) but does not run a signal-averaged 1D data set. Processing occurs automatically (although the data can be reprocessed manually as desired).

Note that, as with standard automation runs, the power levels and pulsewidths retrieved by these setup macros may not be specific for your probes unless you have stored corrected parameter values in either the parameter set or in the final lines of the macro itself.

13.8 Analyte Collector Operation

This section describes how to initialize the Analyte Collector, how to collect analytes, and how to analyze analytes. This section contains the following procedures:

- "Initializing the Analyte Collector," this page
- "Performing Analyte Collection," page 427
- "Performing Analyte Analysis," page 428

Initializing the Analyte Collector

When Cascade is first set up on a new system, or the analyte collector option is first installed, the program must be synchronized with the positions of the bypass valves and the analyte collector loop position.

1. Open the **System Configuration** window, select **Analyte collector on-line**, and close the System Configuration window.
2. Open the Analyte Collector window by selecting **Collector** in the top bar of the Cascade monitor window.
3. Click the **To Column** and **To NMR** buttons two or three times. After the second click, the bypass valve indicators should show the correct status.
4. To synchronize the loop collector valve position between Cascade and the Analyte Collector box, click the **Update Loop Position** button.

   Cascade should stay synchronized unless the loop collector position is changed by using the pane controls on the Analyte Collector.

Performing Analyte Collection

To store separated peaks in an LC chromatogram in individual loops, use the Analyte Collection program.

1. Activate the LC method in **System Control** in **STAR Chromatography**.
2. In Cascade System Configuration, select **Analyte Collection** for the experiment type.
3. In the Cascade monitor window, make sure that **Inhibit Apex Detection** is **not selected.**

4. Click on **Start LC-NMR Run**.

The analyte collection mode of operation, unlike the other modes of operation, does not direct the effluent of the column through to the NMR probe. Analytes can be collected in an environment completely removed from the NMR spectrometer and then analyzed later.

The analyte collection mode uses apex detection to sense peaks in the chromatogram. Analyte collection is started by directing the column output to loop 1. Once a peak is detected, the delay timer starts. When the delay time expires, the 16-port valve is rotated to loop 2, thus trapping the first peak in loop 1. Effluent continues to pump through loop 2 until the next peak is sensed and the delay time is counted. Sample 2 is then stored in loop 2 as the valve is rotated to loop 3.

Performing Analyte Analysis

After the samples have been collected in the loops, you can perform analyte analysis.

1. In the **Batch Scheduler** window, enter the name under which you want the analyte analysis data files to be saved. Do not choose the same name as other data files because these files will be overwritten (refer to "Batch Scheduler," page 450).
2. Open the **System Configuration** window by selecting **Setup** > **Configuration** in the Cascade monitor window.
3. Select **Analyte Analysis** as the Experiment Type.
4. Set the **Run end time** to match the LC method, typically one or two minutes for analyte analysis.
5. In System Configuration, enter the correct loop-to-probe delay time as determined during calibrations
6. Select **Wait for NMR complete**.
7. Close the System Configuration window.
8. Go to the **System Control** page in STAR Chromatography and activate an LC method designed for analyte analysis.

   This method does not perform injections and sets a 1 or 2 minute run time on the pump and detector. This assumes that the same method is used to elute each loop. If different methods are needed, see "Setting Up an Automation Run in STAR Chromatography," page 441.
9. Open the **Analyte Collector** window by selecting **Collector** in the top bar of the Cascade monitor window.
10. Under Loop Analysis Setup, set the following:
    - Enter the number of the **First loop to analyze**
    - Enter the number of the **Last loop to analyzed**.
    - Enter a time in the **Wait between loops** field that allows the LC method to equilibrate. This might be 4 to 5 minutes, especially if different methods are used for different loops.
    - Leave the **Loop inject delay** at **3** seconds to avoid detector rezeroing at the beginning of a loop run.
11. Close the Analyte Collector window.
12. Go to the Cascade monitor window and make sure that **Inhibit Apex Detection** is **not checked**.

13. Click **Start LC-NMR Run**.

When the analyte analysis starts, Cascade moves the analyte collector to the loop position preceding the starting loop position in order for the LC pump to equilibrate and issue a ready signal. After the wait between loops has counted down, the run is started by the LC system and the loop injection delay is counted down. The collector is then switched to the starting loop. This cycle continues until all specified loops have been injected.

The loop wait can be aborted if the pump and detector reach ready state before the wait between loops time has expired.

13.9 Data Processing Methods

Data obtained from LC-NMR experiments can be processed using various methods. These methods are described below. If you select the Auto Processing box in the **LCNMR/STARS** pane, the data is processed automatically at the end of the run, see Figure 119. Auto Processing is valid for all LC-NMR modes except Analyte analysis. If the **Auto processing** option is not enabled or you want to reprocess the data, press the **Process LC-NMR** button in the **LCNMR/STARS** pane. The LC-NMR processing uses the macros `glue, wftlc,`

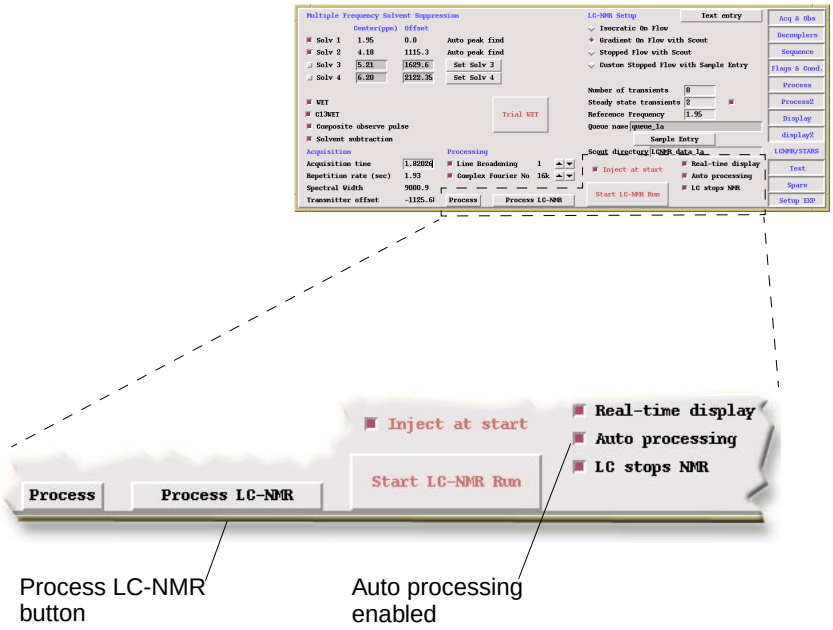


Figure 119. Process LC-NMR Button in LCNMR/STARS Pane

and `wft`. Different data acquisition modes of LC-NMR require different processing. The appropriate processing mode for the type of data acquisition is selected automatically when the **Process LC-NMR** button on the **LCNMR/STARS** pane is pressed. The processing used for each mode of data acquisition is described below.

Isocratic Data

LC-NMR runs acquired using the isocratic conditions end up as an implicitly (using `ni`) arrayed data set in a single experiment. They can then be processed as a *stacked 1D data set* or as a *pseudo 2D data set*.

- To process data as a *stacked 1D data set*, enter **`wft('all')`**
- To process data as a *pseudo 2D data set*, enter **`wftlc.`** The `wftlc` macro adjusts `sw1`, and runs the `setmin` macro, to ensure that the t1-axis is correctly labeled.

Solvent Gradient (Scout Scan) Data and Stopped Flow Data

Runs acquired in the Scout Scan mode end up as a series of 1D spectra stored in separate files. These files can then be combined using the `glue` macro and again processed with either the `wft('all')` or the `wftlc` command.

In this case, the `glue` macro runs automatically; however, the `glue` macro can also be used with time-slice data (acquired using the Stopped Flow with Scout function in the LC-NMR pane.

Manually Processing Gradient On-Flow (Scout-Scan) Data

To process Gradient on-flow data in another experiment, use the following steps:

1. Join the desired experiment (`jexp#`).
2. Load in the file `1.fid` from the scout directory.
3. Change to the parent directory.

   You should be at the same directory level as the scout directory.
4. Set the **Increments** parameter if necessary.
5. Click the **Process LC-NMR** button.

Processing Data from an Aborted LC Experiment

To process data from an experiment of an experiment that was aborted early (with `aa`), use the following steps:

1. Set the **Increments** field in the LC-NMR window to the maximum number of increments completed (or desired to process).
2. Click the **Process LC-NMR** button.

NMRgram

For some applications, using the NMR data to produce an *NMRgram* can be useful. An NMRgram consists of some type of NMR response (a peak height, an integral, the sum of multiple integrals, etc.) as a function of time during the LC run. This can be performed by asking for a projection (using the VNMR menu system) of the entire displayed LC-NMR spectrum, or, if cursors are placed around both sides of a limited region of f2, the projection is for just that region of the spectrum.

Displaying or Plotting LC-NMR Data

All plotting is typically performed by using the button on the **Spare** pane, which is shown in Figure 120.

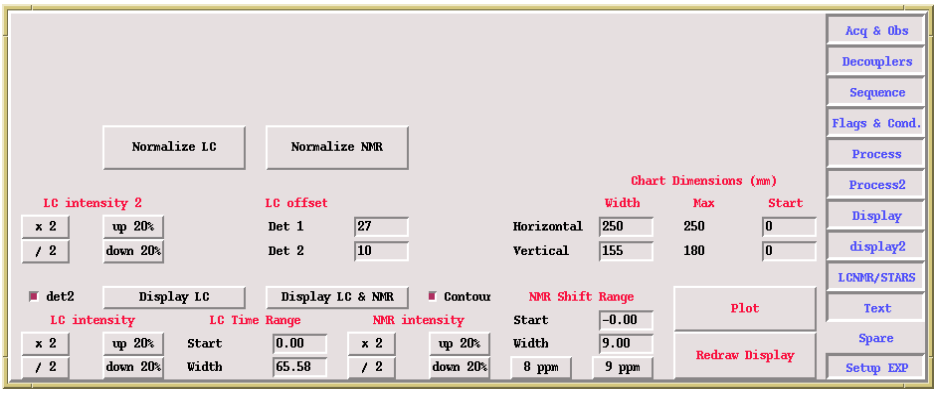


Figure 120. LC-NMR Spare Pane

1D Data Display and Plotting

Spectra processed in the 1D mode can be displayed or plotted using standard stacked spectra commands, such as `dss` and `plww`.

2D Data Display and Plotting

Spectra processed in the 2D mode can be displayed or plotted using standard 2D commands, such as `dconi` or `dpcon`, and with 1D commands, such as `dss` and `plww`.

13.10 Delay Time Calibration

Several delay times must be calculated: one delay for the stop-flow valve and three delays for the analyte collector. For delay time calibrations, a sample of only one of the benzoates is needed. Prepare a sample of the sample concentration 0.5 mg/mL and use 50 microliter injections. Set up an isocratic method of 50% acetonitrile and 50% deuterium oxide.

Calibrating from the LC Detector to the Microflow Probe

The LC-NMR system can perform both continuous on-flow spectral measurements and stop-flow measurements. Continuous on-flow experiments do not require a delay time. A stop-flow measurement is typically used if the sample concentration is too low to be seen on-flow or if more extensive experiments such as COSY, TOCSY, or HMQC are required.

In order to do stop-flow, a detector more sensitive than the NMR is required to allow the LC peak of interest to be detected and stopped in the NMR probe. Between the detector and the NMR probe is a volume equal to the tubing connecting the two detectors, any intervening devices, and the volumes of the flow cells of the detector and the NMR probe.

Because the LC system is forcing solvent through the system at a flow rate set by the user, an LC peak requires a finite amount of time to travel from the detector to the NMR probe. This time, called the *delay time*, is typically is 10 to 12 seconds using the stop flow valve at a flow rate of 1 mL/min with 10 ft of 0.005-inch i.d. tubing connecting the stop-flow

valve to the flow probe. If the analyte collector is plumbed in, the delay time is about 25 to 29 seconds at 1 mL/min. For the ProStar 330, 0.010-inch i.d. transfer tubing must be used; otherwise, it is possible to damage the flow cell. The 0.010-inch i.d. increases the delay times by about 16 seconds.

1. Start the LC system and begin the Stop-Flow Monitor program on the PC.

   If using p-hydroxy methyl benzoate, set up an LC method for 50% acetonitrile/50% D_2O; 1mL/min flowrate; 5 minute run time.

2. In the **Delay Time** field, enter one of the following:
   - **12** seconds if using the stop-flow valve.
   - **25** seconds if using the analyte collector.
3. Set **Hold Time** to 1 minute. Make sure **Wait for NMR** is **unchecked**.
4. Prepare a sample that provides an easily observable NMR peak under continuous on-flow conditions. You should be able to see a peak with `nt=4` or less.
5. Set up the NMR appropriately for LC-NMR. Set **`nt=4`** and set **`ni`** to a large value that allows you to observe at least one of the LC peaks that elutes off the column and through the probe. Expanding the region around where you expect to see an NMR peak is helpful; however, you can expand this region after data is collected.
6. Start the LC system. Start the NMR before the peak reaches the probe.
7. Monitor the NMR as the LC peak elutes through the probe. As the peak moves into the probe, the signal increases.
8. Enter **`wft('all')`** to process the data. Enter **`dssh`** to display the data. If your calculated Delay Time is correct, you should see a profile similar to Figure 121 after you enter `dssh`.

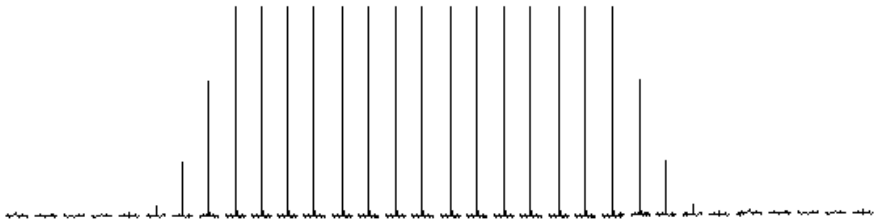


Figure 121. Profile Showing Delay Time is Correct

The profile shown in Figure 121 is the result of the Stop Flow Monitor program switching the stop flow valve to isolate the sample in the probe exactly on the peak apex, when the highest concentration of sample is in the probe. The flat-topped appearance is the result of the hold time keeping the valve switched for 1 minute.

If the delay time is too short, the profile looks like Figure 122.

The profile shown in Figure 122 is the result of the Stop Flow Monitor program switching the stop flow valve before the peak apex reaches the probe cell. Consequently, the system holds the low concentration of sample in the probe for the 1 minute period. When flow resumes to the probe, the apex elutes through and is observed on the NMR. Fix this by increasing the delay time. You can estimate the amount of increase required from the time required from each spectrum and the number of spectra taken from when the valve closes to when the apex passes through the probe.

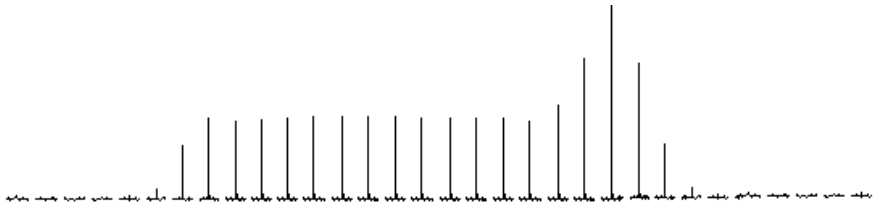


Figure 122. Profile Showing the Delay Time is Too Short

If the delay time is too long, the profile looks like Figure 123.

Figure 123. Profile Showing the Delay Time is Too Long

The profile shown if Figure 123 is the result of the apex peak passing through the probe cell and the Stop Flow Monitor program causing the stop flow valve to switch as the peak is leaving the probe cell. Fix this by decreasing the delay time.

Calibrating from the LC Detector to the Analyte Collector

The NMR system is not needed for calibrating from the LC detector to the analyte collector. It is best to use an isocratic run for this procedure.

1. Open the **System Configuration** window of the Cascade program.
   - Make sure that the **Analyte collector on-line** box is **selected**.
   - Select **Analyte Collector** for the **Experiment Type**.
   - Make sure **Wait for NMR Complete** is **not selected**.
   - Enter **5** seconds for the **loop fill delay**.
   - Check to see that the calibration is starting in loop 1.
   - Make sure the **Inhibit apex detection** is **not selected**.

   The correct delay time does not appear until a run is started.
2. Start a run with the benzoate sample. Use a 50% acetonitrile/50% D_2O method with 1mL/min flow rate. The first sample is stored in loop 1 with the first loop fill delay time of 5 seconds.
3. Repeat steps 1 and 2 using different delay times, in 1-second increments, up to 11 seconds.

   Store successive samples in successive loops; this process is automatic unless there is user intervention. This process fills 7 loops. Monitor the apex intensity of the signal stored in each loop.
4. Do an analyze loops cycle.

a. In STAR Chromatography, create an **isocratic method** of 50% acetonitrile/ 50% D_2O with a **2 minute run time**.

b. Inhibit injection by not setting the relays in the pump section of the method.

c. **Save the method** and then go to **System Control** and **Activate the method** and wait for the ready lights on the pump and detector.

d. In the **System Configuration** window of Cascade, check **Analyte Analysis**.

e. Set the **Run end time** to **2 minutes**.

f. In the **Loop analysis** section of the Analyte Collector window of Cascade, set the start of analysis to **loop 1** and finish with loop 7. Set a **delay of 3 seconds** and a wait of 4 minutes.

5. In the **Main menu** (data acquisition and display) click on **Start Run**.

6. Each loop should be automatically emptied in turn and the UV spectrum recorded. Monitor the apex intensity of the eluted compounds. The correct delay time is the shortest time necessary for maximum intensity. Typical values at 1 mL/min are between 6 and 10 seconds. Enter the value in the configuration window of the LC-NMR stop-flow program.

Calibration from the Analyte Collector to the Microflow Probe

Once the delay time from the UV detector to the analyte collector has been calibrated, it is necessary to calibrate the delay time from the analyte collector to the flow probe.

1. Load about ten loops with the methyl benzoate sample. This requires 10 separate injections.
   - Use the delay time calibrated for loop loading.
   - Use an isocratic method: 50% acetonitrile/50% D_2O. 5-min. end time, 1 mL/ min.
   - Inject 25 mg on-column (50 µL of 25 mg/50mL standard)

2. Set the system up for analyte analysis:

   a. In **System Control** of STAR Chromatography, activate the **LC method** used for analyte analysis in the previous calibration.

   b. In **System Configuration** of Cascade, select **Analyte Analysis**.

   c. Set the **hold time** to **1 minute** and make sure that the system is not waiting for the NMR to complete.

   d. Set the **End time** to **2 minutes**.

   e. Start with a **delay time** of **10 seconds**. This assumes that the LC method is at 1 mL/mi).

   f. Make sure that the **Inhibit apex detection** is **not selected**.

   g. Go to the analyze loops section in the analyte collector window, set the wait time between runs to 3 to 4 minutes. Start and end with loop 1.

   h. Click on **Start Run** in the main menu to start analyte analysis.

   After the wait time is counted down, loop 1 is emptied and a peak is detected in the LC/NMR stop-flow program. The delay time is counted down and the system stops while the NMR acquires data.

3. After the hold time has expired, the LC-NMR run continues.

4. After the NMR and Cascade finish, change the delay time to 11 seconds in the **System Configuration** menu.
5. In the **Analyte Collector** window, set the first and last loop to be analyzed to 2.
6. When the LC pump and detector are ready, click **Start Run**. Loop 2 is emptied, and the new delay time is invoked after a peak is detected.
7. Increment the delay time for each subsequent loop and restart analyte analysis for that loop. The correct delay time is the time that results in NMR data as shown in Figure 121. Typical values are between 10 and 15 seconds at 1 mL/min.

Note: Changes in the length or i.d. of the connecting LC tubing require the delay times to be recalibrated. Changes in flowrate also affect the delay time but new delay times can be calculated. For example, half the flowrate results in a two-fold increase in the delay time.

New Solvent Concentration

For a new solvent concentration, enter the macro `getwet`. The `getwet` macro runs `setscout ga setwet ga`, which acquires an `nt=1` scout scan and a solvent-suppressed spectrum of the new solvent mixture.

13.11 Chromatography for LC-NMR

This section describes the chromatography side of LC-NMR and provides some information on how to use the STAR chromatography program on the PC. This section also discusses the standards used for LC calibration and provides some typical chromatograms for comparison. The following is an outline of this section:

- "Standards Used for LC-NMR Chromatography," this page
- "Starting the LC pump," page 436
- "Setting Up STAR 5.31," page 436
- "Building a Method in STAR 5.31," page 438
- "Printing a Chromatogram," page 440
- "Using the Cascade Program," page 443
- "Typical Chromatograms," page 441
- "Setting Up an Automation Run in STAR Chromatography," page 441
- "Hints for Good LC-NMR Chromatography," page 442

Standards Used for LC-NMR Chromatography

The following three compounds were purchased from Aldrich and used as standards for LC-NMR:

- Methyl-4-hydroxybenzoate (M5,010-9)
- Ethyl-4-hydroxybenzoate (11,198-8)
- Propyl-4-hydroxybenzoate (P5,335-7)

A standard solution was prepared by dissolving 25 mg of each compound in a 50-mL volumetric flask in 25 mL of acetonitrile and filling to the mark with a deuterium oxide. The sample volume injected is 50 microliters.

The LC column used is a Varian 15-cm, 4.6-mm i.d. C18 column (Part No. 03-912159-42). A column is shipped with each system.

Proprionitrile-free acetonitrile is purchased from VWR (Omnisolve brand). Glass-distilled acetonitrile is manufactured by EM Science (Part No. AX0142-6).

Starting the LC pump

Before starting LC-NMR experiments, the system should be equilibrated by pumping solvents through the column and the Microflow probe.

1. Press the **STATUS** button on the front panel of the LC pump.

   If the REMOTE LED is lit, the system is still under remote control. Press the **LOCAL** button and then the **RESET** button.

2. After the pump status is displayed, press the **%ABC FLOW** button.
3. Use the arrow buttons to change the mobile phase proportions of A, B, or C.
4. Set the proper flowrate.
5. After the parameters have been adjusted, press the **RESET** button **twice** and the new parameters will appear.
6. Press the **PUMP** button to start the pump.
7. Let the pressure stabilize before starting any experiments.

CAUTION: **Never exceed 2.0 mL/min flow rate through the probe. High pressure will damage the flow cell and tubing in the probe.**

Setting Up STAR 5.31

This section describes how to set up STAR 5.31 software.

- "Loading STAR 5.31," this page
- "Building a Method in STAR 5.31," page 438

Loading STAR 5.31

1. Install the STAR 5.31 software from the CD-ROM.
   a. Insert the STAR 5.31 CD-ROM and click **INSTALL**.
   b. Select **STAR Chromatography Work Station**.
   c. Read the license agreements and click **Agree**.
   d. Enter **name**, **company**, and **serial number** (from bright yellow sheet).
   e. Install the software into the directory **`C:/STAR`**.
   f. In the LC Components window, select **Example Data** file and **Advanced Applications**. If you have a 9065 detector, click on **9065 driver box**. Click **NEXT**.
   g. Answer **No** to the question `Do you want STAR tool bar to start automatically?`
   h. Press RETURN and EXIT.

   After the software is loaded, a STAR icon appears in the left side of the screen.
2. Load STAR Update #2.

a. Insert the first of the three floppy disks.

b. Click on the **FLOPPY A** icon to open files.

c. Double-click on the blue computer icon labeled **set up**.

d. If necessary, enter **A:** for the path.

The software begins to load.

e. At the prompt, eject the disk and insert the second disk. A:\ is the correct path.

f. At the prompt, eject the disk and insert the third disk. A:\ is the correct path.

3. If using the ProStar 330, install Polyview 2000 from the CD-ROM. This requires a superuser password. See description in setting up 330.

4. Disable modules that are not being used from the STAR toolbar.

5. Set up the STAR software.

a. Click on the **STAR Tool bar** icon (left side of the screen).

b. In the right side of the tool bar, at the top of the screen, click the right mouse button and select **Module Disable/Enable** from the menu that appears.

An upper and lower box appears. All possible modules made by Varian are enabled in the top box.

c. Highlight the module that you have *not* installed on your system by clicking on their names. After the modules are selected, click the **disable** button.

The modules that you have disabled appear in the lower box where you can enable them later if necessary. The modules that you have left enabled appear in the upper box.

d. Click **OK** when you are finished.

e. Open **System control** (upper left computer icon on the STAR tool bar).

f. If you are asked if you want to update some methods, continue to click **OK** until it is finished.

Four gray boxes open that represent the four systems that can be run from your computer. The remote orange light on each module lights up if communications between the computer and module is established. Under the four gray boxes are icons representing your modules.

g. Place the icons for your modules into Instrument One in the upper left side.

h. Double-click on one of the module icons. A small control window for each module is created. Each window contains all of the controls for the particular module, such as *pump on/off*, and so on.

6. Set up the LC instrument from the System Control icon. Drag the icons for the pump and detector into instrument 1 as shown in Figure 124. Double click on one of them when the icon outline turns black. An image of the front panel of the module should then be displayed.

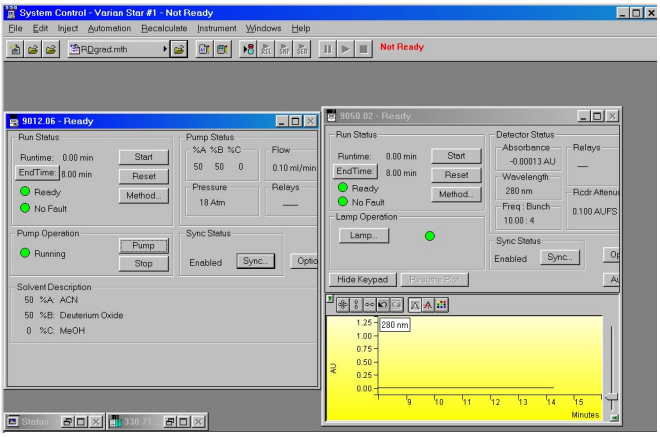


Figure 124. STAR System Control Window

Building a Method in STAR 5.31

This procedure describes building a method in STAR 5.31.

1. Enlarge the pump and detector windows to monitor the pump and detector states.
2. Click on the **method builder icon** (second from the left) in the STAR toolbar, similar to Figure 125. Choose **create a new method**. A wizard pops up.

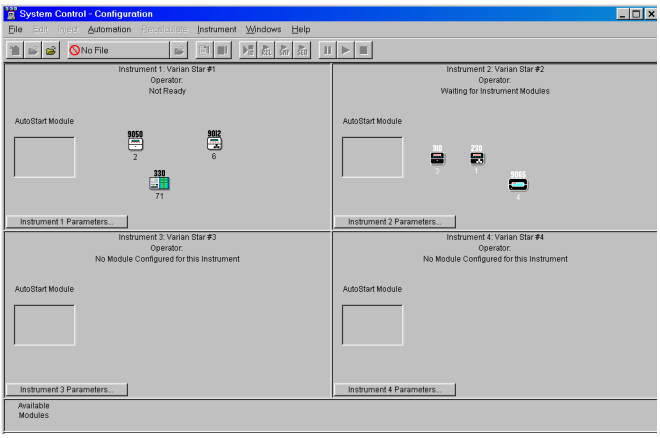


Figure 125. STAR Configuration Window

3. Choose **Next**. In the new window that pops up, select a configuration for instrument 1. Both the pump (9010, 9012, or ProStar230) and detector (9050, 9065, ProStar310, or ProStar330) modules should be listed on the right-hand side.
4. Click **Next**. Another window pops up. Make sure that the correct detector is listed.
5. Click on **Next**. A window pops up listing sections for post-run processing. Choose both data handling and standard reports.
6. Click on **Next**. A final window pops up. It should list the pump, detector, and both data handling and standard reports.
7. Click on **Finish**. A window pops up asking you to choose between opening a method or creating a new method.
8. Click on **Cancel**. The Method Builder screen with all the sections and subsections is displayed. Follow the instructions in "Setting Up Method Parameters," page 439 to set up the method parameters.

9. When finished setting up the method, open the **File** menu and choose **Save As**. Enter a name and click on **Save**. Open the **File** menu again and select **Exit**.

Setting Up Method Parameters

1. Click on method parameters under the 9012 or ProStar230, similar to Figure 126.
2. Set up the pump method as needed. Figure 126 shows an example of a gradient method.
3. Move to the detector module click on Method Parameters.

   Figure 127 shows an examples of ProStar 310 and 330 methods.
4. To set the display wavelength in the ProStar 330, go to the peak sense time program option of Polyview 2000. select the desired wavelength. This should update the channel 1 heading.
5. To convert results files to ASCII in both the 9050/ ProStar 310, go to the Print Options page of Standard Reports. Select the Convert results to ASCII box. (Unselect the Automatic printing of reports & chromatograms if you don't want a STAR printout of each run or if you are not connected to the printer).

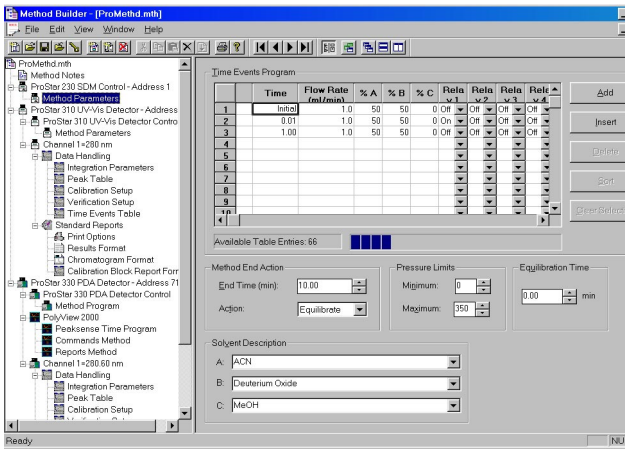
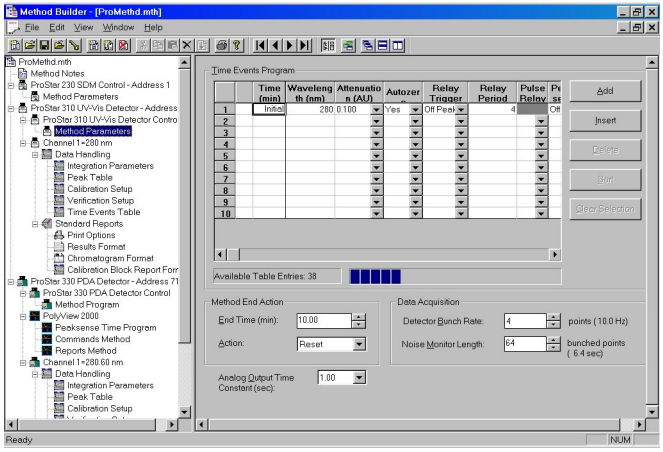


Figure 126. STAR Pump Method

ProStar 310 Detector Method Window



STAR ProStar 330 Detector Method Window

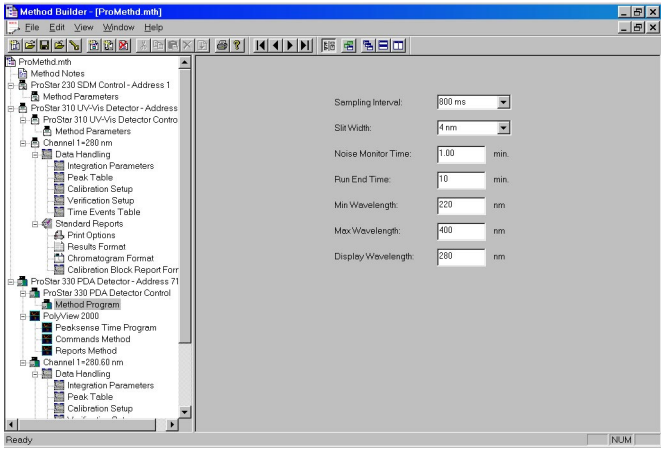


Figure 127. Detector Method Windows

6. To include a run log that has the pump and detector method parameters, go to the Results format page under Standard Reports and select the Run log box.
7. After you are finished entering parameters, select Save or Save As from the File menu.
8. Select Exit from the File menu.
9. If necessary, click on the System Control icon to return to the System Control window.
10. Open the file menu and chose activate method. A list of method files is displayed. Choose the correct method and click on **open**.
11. For the method to download to the pump, the pump must be running. Click on the **pump** button on the panel display.

Activating a Method

1. From the System Control window, open the **File** menu and choose **activate method**. A list of method files is presented. Choose the correct file and click on **open**.
2. The new method should have been downloaded (check the endtimes and wavelength of the pump and detector windows) and the method name should now be listed at the top of the page.

Printing a Chromatogram

1. From the STAR tool bar, select the **Interactive Graphics/Data Handling**.

   Select the appropriate **data file**. STAR 5.31 saves detector files with the following naming schemes:

   - 9050 detector files are saved under the name `9050.03xxx`.
   - ProStar 310 detector files are saved under `310.xxx`.
   - ProStar 330 detector files are saved under `330.xxx`. To view UV spectra, use PolyView.
   - 9065 detector files are saved under `9065.xxx`. To view UV spectra, use PolyView

   Click **Open Files** when finished.

   A data file should now be displayed.
2. From the **File** menu, select **print**. Click **OK**.
3. To print results, go to the **Results** menu, select the data file. You can choose the View Results Only option. (If no data file report is available, choose the **reintegrate now** feature. A new window with the integration results is displayed.)

   A results format window appears. If the run log was not appended, you can do so now by going to **Options** menu and selecting **Results**
4. In the run **documentation section**, click on **run log**. This appends the method parameters actually used to acquire the run. Click **OK** when finished.

   The Results window should reappear.
5. From the **File** menu, select **Print**, check both the **chromatogram and results** options. Click **OK**. The chromatogram and results should now be printing.

Typical Chromatograms

Figure 128 shows a typical chromatogram of an isocratic run of the three benzoates with the 9050 UV-Vis Detector. LC conditions are described in the isocratic methods section.

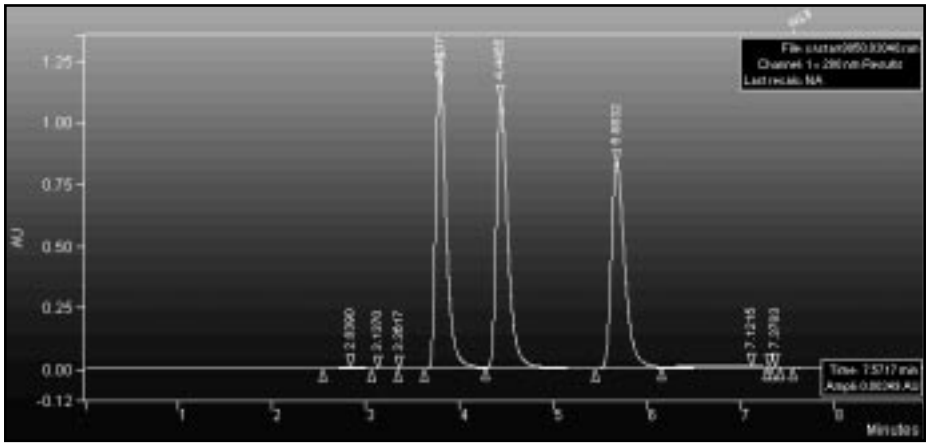


Figure 128. Isocratic Run of the Three Benzoates with the 9050 UV-Vis Detector

Setting Up an Automation Run in STAR Chromatography

For samples stored in the analyte collector under gradient conditions, a sequence file must be set up in STAR Chromatography so that each loop can be eluted using the LC conditions that the sample was stored in.

1. Record the mobile phase composition as a sample is stored in a loop.
2. Create an LC isocratic method for each different composition. Use short run times and inhibit injection. Keep the method names simple, such as `loop1.mth`, `loop2.mth`, etc.
3. In **System Control**, select **File** > **sequence file** > **new**.

   A Sequence List appears, similar to Figure 129.
4. Enter data information for each loop.
   - The action to use is inject.
   - The method used is different for each loop, based on the solvent composition used to store the sample in the loop.
   - The sample list/log can be the default value.
   - You can add an extra line at the end of the sequence to turn off the pump at the end of the sequence run.
5. Click **Begin**. The sequence downloads the method but does not start until Cascade is started.
6. Go back to the Cascade program and start an analyte analysis run. Make sure to set a **wait time** of **at least 4 minutes** between loops.

   For analyte analysis runs of 12 hours or longer, use a delay time between runs of sufficient time for the detector to write the data file. This can be up to 30 minutes or more for a 9065 data file.

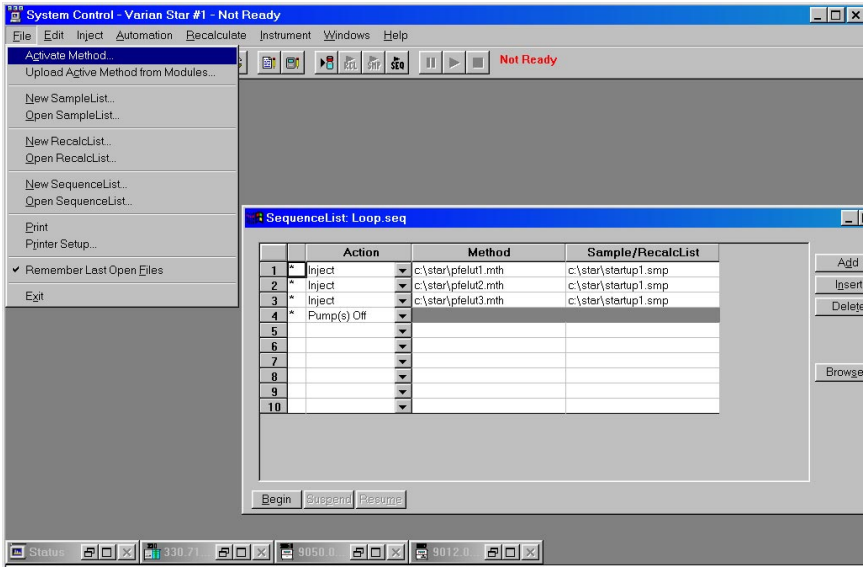


Figure 129. STAR Sequence List Window

7. Click Start run in Cascade.

   The analyte analysis run starts by counting down the *Wait between loops*. If the pump and detector are ready, click on the **Abort wait** button. Cascade and STAR should then start with the automation run.

Hints for Good LC-NMR Chromatography

This section outlines some hints for optimal LC-NMR chromatography.

- To maintain spectral homogeneity in the Microflow probe, LC gradients should be no steeper than 2% per minute at 1 mL/min.
- Mobile phases that are well suited to LC-NMR chromatography include:
  - acetonitrile
  - deuterium oxide
  - phosphate buffers
  - ammonium acetate buffers
  - formic acid buffers
  - trifluoroacetic acid in acetonitrile or deuterium oxide
  - acidic methanol
- Methanol/buffer combinations can be troublesome because slow proton exchange leads to broad NMR OH peaks.
- Ethanol is a poor choice of organic solvent because it has so many protons; the region of signal suppression in the NMR is quite large when compared to methanol. Triethanol amine modifier is also a poor choice for the same reason.
- Retention times in deuterium oxide buffers are somewhat longer than in the corresponding aqueous buffer.

13.12 Cascade LC-NMR Stop-Flow Program

The total LC-NMR user interface includes the Cascade LC Stop Flow Monitor program and the VNMR interface. This section describes the Cascade program windows:

- "Using the Cascade Program," page 443
- "Cascade LC-NMR Monitor Window," page 445
- "System Configuration," page 447
- "Time Program," page 450
- "Batch Scheduler," page 450
- "Analyte Collector Window," page 451

Cascade is a Windows 95 program designed to control an LC system interfaced to a Varian NMR system. Cascade is used to detect elution events using the output of the LC UV detector and control the sampling interface to the NMR Microflow probe. Cascade also signals the NMR to begin a data acquisition cycle after allowing for the delay in moving the sample from the LC into the probe. Several types of experiments are possible depending on the needs of the sample: on-flow, stopped flow, analyte collection, and analyte analysis.

Using the Cascade Program

Cascade was developed for use with Windows 95, and an installation utility is provided that installs and registers the program by using the Add/Remove program dialog box in the Control Panel group. Cascade can also be uninstalled using the same utility. The program and associated drivers come on two floppy disks.

The Setup utility checks the system for files that require updating and then decompresses the Cascade files and registers the application. The Setup utility also adds information about the drivers to the `System.ini` file located in the Windows directory. The previous `System.ini` is saved as `System.old` and should be used to replace `System.ini` if Cascade is uninstalled.

To Initialize Cascade

1. To start Cascade, click on the Windows 95 **Start** button, open the **Programs** menu, and choose **Cascade**.

   Alternatively, open the Windows Explorer window and navigate to the directory `C:\Program Files\Cascade`. Click on the executable file in this directory

2. Once Cascade starts, a window pops up with the status of the A/D board. Click **OK**.

3. Open the **Set Up** menu and choose **Communications**. Set the **loop increment delay** to **50** (cycles) and the **loop read delay** to **10,000** (cycles). Then close the window.

To Run Cascade

- Select **Start** > **Programs** > **Cascade**

A dialog box appears indicating that the data acquisition card has been opened by the driver software and configured. Cascade appears after this dialog has been cleared. If a dialog box displaying "`Error loading DLL!`" appears, the software drivers have been incorrectly loaded or may have been deleted by another program installation or removal.

To Start a Run Manually

1. Open the System Configuration window by clicking on **Setup** > **Configuration**.
2. Enter the desired run parameters in the System Configuration window.

   For the three benzoate mix, set the run time to 8 minutes and the flowrate to 1.0 mL/min. Set the detector sampling rate to 0.5 seconds.
3. Close the System Configuration window.
4. Click the **Start Run** button.

   The current display is cleared and display and run parameters are loaded from the System Configuration window.

   The first pane in the status bar on the bottom of the Monitor window displays the message "`Run in Progress`" and the elapsed time is displayed in the second pane.

To Trigger a Run by the NMR Console

A run can also be triggered from the NMR console once the run parameters are set up.

1. Open the System Configuration window by clicking **Setup** > **Configuration**.
2. Enter the desired run parameters in the System Configuration window. To have the NMR started when a peak is detected and to hold until the NMR is finished, select **Wait for NMR Complete**.
3. Select **Watch for NMR start**.
4. Select the desired display defaults:
   - To put a marker at the point in the chromatogram where Cascade is holding for NMR data acquisition, select **Markers at transfer delay**.
   - To put a marker at the apex of the peak that triggered the stop-flow, select **Markers at peak apex**.
5. Close the System Configuration window.

While the run is in progress, the Start Run button is grayed out.

If the threshold is set correctly and Inhibit Apex Detection is *not* checked, Cascade detects peaks automatically as they come through the detector.

When a peak is detected, the stop-flow sequence is triggered and Cascade counts down the sample transfer delay time to allow the peak to reach the Microflow probe. Once the sample is in the Microflow probe, Cascade switches the stop-flow valve or the valve on the analyte collector so that the probe is isolated. Cascade also stops the LC pump.

Once the peak is in the Microflow probe, Cascade holds for NMR data acquisition. Cascade either counts down the NMR hold time or waits until the NMR sends a *done* signal. NMR holds can be terminated manually by clicking the Abort Stop Flow button.

To Stop on Peaks That Do Not Reach Threshold

Peaks that do not reach the threshold value do not trigger the stop-flow sequence. You can stop on these peaks during the run.

- To stop on a peak and invoke the delay time, click the **Stop Flow** button.

  This button invokes the stop flow sequence and counts down the delay time; Cascade then switches the stop flow valve and stops the LC pump.

- To stop on a peak immediately, *without the delay time*, click on the **Hold Run** button.

  Cascade stops immediately without any delay time countdowns. This feature is useful for manual time-slicing, especially through coeluting peaks.

To Detect an Analyte with No UV Signal

The Time Slice button allows you to detect an analyte with no UV signal if you know the approximate elution time.

- Click the **Time Slice** button to stop the LC immediately and hold for the NMR.

When the NMR is finished, the run continues for a fixed interval (set by Time slice width in the System Configuration window) before pausing for another NMR hold. Time slices are indicated on the chart using a vertical blue line. The time slice feature can be started and stopped manually using the Time Slice button.

To Terminate a Run Before the End Time

- To terminate a run before the end time specified in the System Configuration window, click on the **Stop Run** button.
- To terminate the *analyte analysis experiment*, click on the **Stop Analysis** button.

The data file is automatically saved to the disk under the name in the Batch Scheduler. The data file can be saved manually from the file menu in the Monitor window. If the experiment type is an analyte analysis, Stop Run terminates the present loop being analyzed and proceeds to the next loop.

At the end of the specified run time, Cascade saves the data to the hard disk and activates the mouse cursor for working with the chart display. Labels can also be added to the plot, using the right mouse button. Data files can be saved using the File menu commands.

Cascade LC-NMR Monitor Window

The Cascade monitor window, shown in Figure 130, is the primary window for working with the Cascade program. The monitor window provides a display of the LC detector output during the experiment and various informational messages on the status bar at the bottom of the window. The window also contains the controls used during the experiment, such as Stop Run and Abort Hold, and controls for the detector output display.

The Cascade monitor window is the central window during an LC-NMR experiment. The display area shows a plot of the detector output, which updates according to the sampling rate selected on the System Configuration window.

The plot has axes in time (decimal seconds) on the x-axis and signal (mV) on the y-axis. The data points are smoothed as they are acquired. The y-axis scale and offset of the display can be changed during the run without affecting the default values by using the controls to the right side of the display.

During a run, status messages appear in the status bar at the bottom of the window. In addition, several panes display the times of various operations such as run elapsed time and NMR hold time.

Cascade consists of the main viewing window, known as the Monitor window and five satellite windows:

- System Configuration
- Time Program

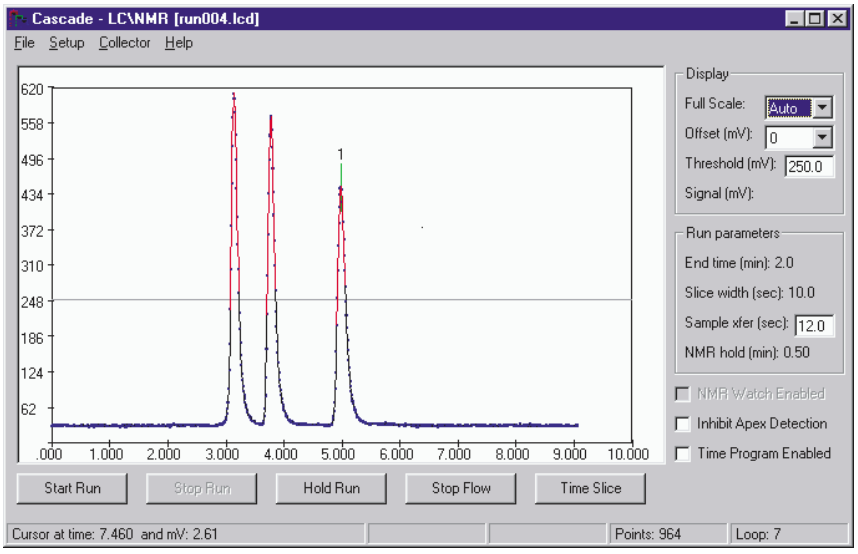


Figure 130. Cascade Monitor Window

- Analyte Collector
- Batch Scheduler
- Communications

In the menu bar at the top of the Cascade Monitor window are the File, Setup, Collector and Help menus.

File Menu

The options under the file menu are:

- Open – Opens saved data files.
- Save As – Saves LC-NMR stop-flow runs.
- Copy Chart to Clipboard – Copies the chromatogram to the clipboard.
- Print Window – Prints the entire window, including run parameters.
- Print Chart – Prints the chromatogram
- Printer Setup – Chooses and configures a printer.

Inhibit Apex Detection

Checking the Inhibit Apex Detection box turns off the apex detection mechanism. This can be used when solvent fronts are present in the chromatogram or as a means to not stop on peaks that are not of interest.

Cascade Chart Display

During the run, you are limited to changing the plotting values in the Display field. The Full Scale and Offset values are in fixed increments and selected from the drop-down list.

The Threshold value sets the signal trigger level for peak detection and can be modified from the value entered in the System Configuration window.

- To change the Threshold value during a run, enter the new value in the **Threshold** field and press the **Enter** key.

When the run is completed, the mouse cursor is activated and becomes a flexible cross when the cursor is within the boundaries of the chart. The position of the cross on the chart is displayed in the first pane of the status bar at the bottom of the window. The cross can then be used to read the time or signal of a particular event.

- To zoom a selected region and fill the chart, **hold the left mouse button and drag** around the region of interest.
- To return to full scale, **double-click** in the chart.
- To add annotation to the chart events, **click the right mouse button** at the **desired location in the chart**.

  A dialog opens for adding annotations to the selected chart events. Be sure to position the chart cursor first at the desired location before double-clicking. Up to ten label characters can be entered in the dialog box. The label appears when the dialog is closed. The labels and NMR hold markers are saved with the data file and are restored if the file is opened.

Run Status and Other Information

The status bar at the bottom of the Monitor window contains five panes:

- The first pane displays general status messages about program operation and to alert the user about active Time Program events.
- The second pane displays the elapsed run time.
- The third pane displays the presently active delay time from sample transfer, NMR hold, or loop start (in analyte analysis mode).
- The fourth pane shows the status of the peak tracking algorithm as it follows the data points.
- The fifth pane indicates the present loop position of the analyte collector.

System Configuration

The System Configuration window is opened from the Setup menu. The window is used to set up the type of experiment and the parameters for the run or series of runs, such as run time and sample transfer delay. The System Configuration window allows the present setup to be saved as a default and sets the analyte collector option. The main fields in the System Configuration Window, shown in Figure 131, are

- Experiment type
- Run start options
- Display defaults
- Run default
- Advanced options

Experiment Type

The experiment type field provides choices for the type of LC experiment.

- On-Line/Stop Flow – For stop-flow LC runs. Stop-flow runs can be changed to on-flow runs by clicking on Inhibit Apex Detection in the Cascade monitor window.
- On Flow – For on-flow LC runs.

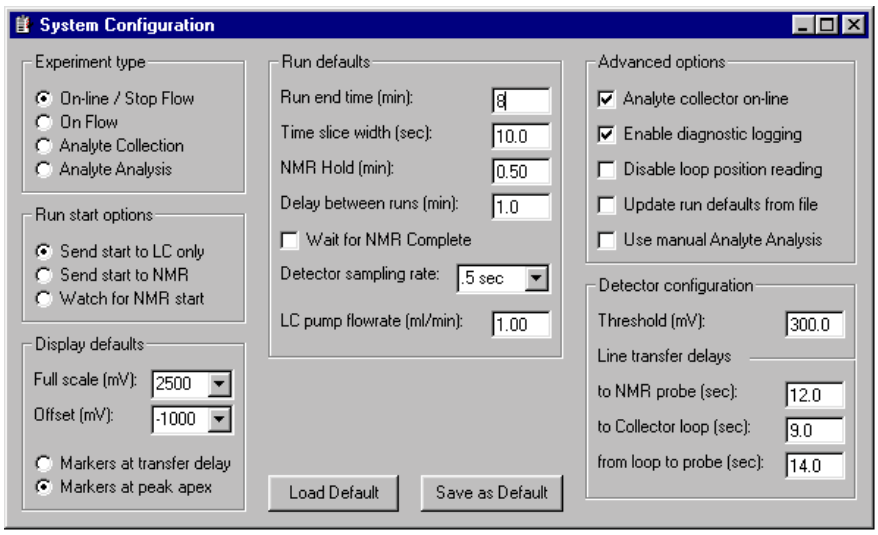


Figure 131. Cascade System Configuration Window

- Analyte Collection – For analyte collection mode. This directs the proper signaling to the analyte collector valve and switches.
- Analyte Analysis – For the analysis of samples stored in the loops.

Run Start Options

After the type of experiment has been chosen, the run start options should be set. You can choose to have the LC-NMR experiments started by the NMR console or by the Cascade program.

- To start Cascade and STAR chromatography from Start Run button in Cascade, from the START button on the LC pump, or from the START button on the 9050 LC detector, select **Send start to LC only**.

  This option allows you to start the LC system if it is not interfaced with the NMR system, such as during method development or analyte collection.
- To start an NMR run from the PC, select **Send start to NMR**.
- To start the LC-NMR experiments and Cascade from the NMR console, select the **Watch for NMR** option.

  The system then waits for an inject signal from the NMR. The downside to this option is the other programs on the PC may run more slowly while the system is waiting for the NMR start signal.

Display Defaults

You can set the parameters that control how the data from the UV detector is displayed in the Cascade monitor window.

- Full scale and Offset – Make adjustments to listed values. Both of these settings can be overridden from the Cascade monitor window while a run is in progress. The display is updated on the fly.
- Markers at transfer delay – Hash marks are drawn at exactly that point in the chromatogram where the pump is stopped during a stop-flow measurement. This is usually not on the peak apex, but rather the delay time past the peak apex.

- Markers at peak apex – Markers are drawn at the apex of the peak that triggers stop flow.

Run Defaults

The run default values set up the basic parameters of the experiment and cannot be changed (with the exception of the Threshold setting) from the Cascade monitor window once a run has been started. The following is a list of descriptions for the Run defaults field.

Run time	The length of the run time, in minutes.
Time Slice Width	The interval of time slices, in seconds.
NMR hold	The time the NMR should hold during stop flow or analyte analysis, in minutes. A hold time of 1 minute is often set during delay time calibrations.
Threshold	The level of the threshold, in mV, above which peak detection is invoked during stop flow, analyte collection, and analyte analysis.
Transfer line delay	The delay time, in seconds, between the UV detector and the Microflow probe for stop flow runs. If analyte analysis is being done, enter the value of the transfer line delay from the loops to the Microflow probe.
Collector loop fill delay	The delay time, in seconds, between the UV detector and the analyte collector loops.
Delay between runs	The delay between analyte analysis runs.
Wait for NMR to complete	Whether or not the NMR console resumes experiments after it is finished collecting data during a stop-flow run, or if signals need to be sent to the NMR once a peak is detected in the chromatogram and a stop-flow run is started. The Wait for NMR complete option overrides the NMR hold option.
Detector sampling rate	The rate, in seconds, that the detector samples data. For short chromatographic runs with narrow peaks choose a high sampling rate (e.g., 0.25 sec). For longer runs, choose a value of 1 or 2 sec per data point.
LC pump flow rate	The flow rate used in the LC method. If this flow rate changed from a previous value, the delay times are automatically updated.

Load Default and Save as Default

- To save the current System Configuration settings as the default settings, click the **Save as Default** button.
- To use the values from a previous run, **open the desired data file** (File menu in Cascade monitor window), and then click the **Load Default** button in System Configuration.

  The values from the data file fill the System Configuration window and are used for the next experiment.

Advanced Options—Setting the Analyte Collector

After the analyte collector is installed, select **Analyte collector on-line**. Make sure the stop-flow valve is disconnected from the plumbing and signal cables, i.e., the P5 connector.

Time Program

The Time Program window, shown in Figure 132, is used to set up a series of events to occur during a run.

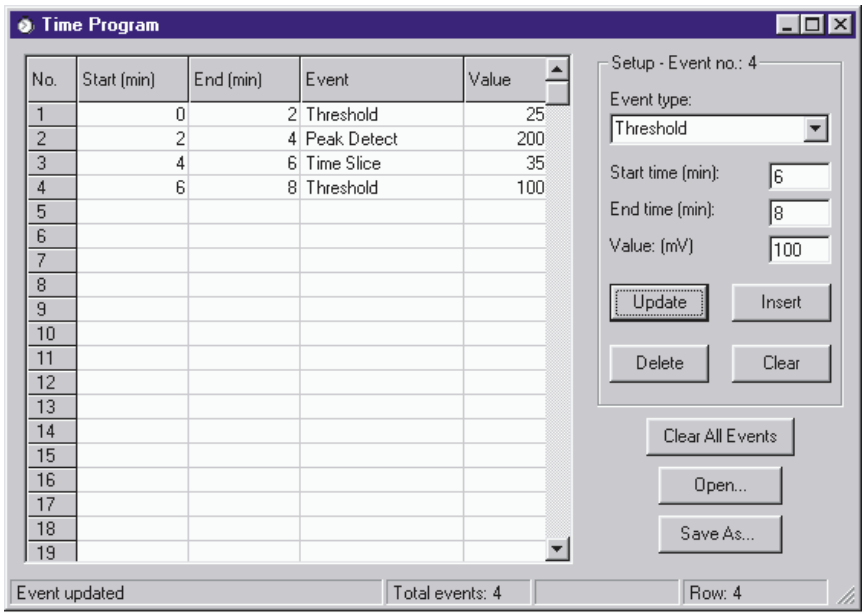


Figure 132. Time Program Window

The Time Program enables you to trigger events or change run parameters at specific times during the experiment. The program can inhibit peak detection, change the peak detection threshold and start and stop time slicing.

When opened, the Time Program window loads the default event table. The events are placed into the event table. If more than 20 events are in the table, the scroll bar along the right side can be used to view them.

- To edit an event, **click on the even**t, change the **Start time**, **End time**, or **Value fields**, and click **Update**.
- To clear the entire table, click the **Clear All Events** button.
- To clear event parameter, **click on the event** in the list and then click **Clear**.
- To delete an event, **click on the event** in the list and then click **Delete**.
- To insert a new event, **click in the list** where you want the event inserted and then click **Insert**. All events, including the selected one, move down one place in the table and an empty event is inserted in the present place.
- To save the event table, click **Save As**.

Clicking **Done** opens a dialog box asking the user whether to enable the time program for the next run. The Time Program window is then closed. If the time program is enabled, the Time Program Enabled box in the Cascade monitor window is checked.

Batch Scheduler

The Batch Scheduler window, shown in Figure 133, can be used to automatically name run files.

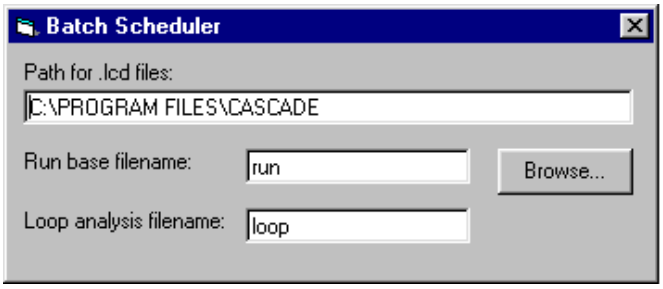


Figure 133. Batch Scheduler Window

- To open this window by selecting **Setup** > **Batch Schedule** from the Cascade monitor window.
- To name a series of files, enter a name in the **Run base filename** field. The first run uses the name appended by *001*. Subsequent runs use the same base name appended sequentially

Analyte analysis runs can also be named with the batch scheduler. Do not them the same name as the Run base filename.

Communications

The Communications window (see Figure 134) can be used to manually transfer `*.lcd` data files to the Sun workstation or to have them automatically transferred. Also, the Communications window is used to set the loop increment delay to 50 and the loop read delay to 10,000.

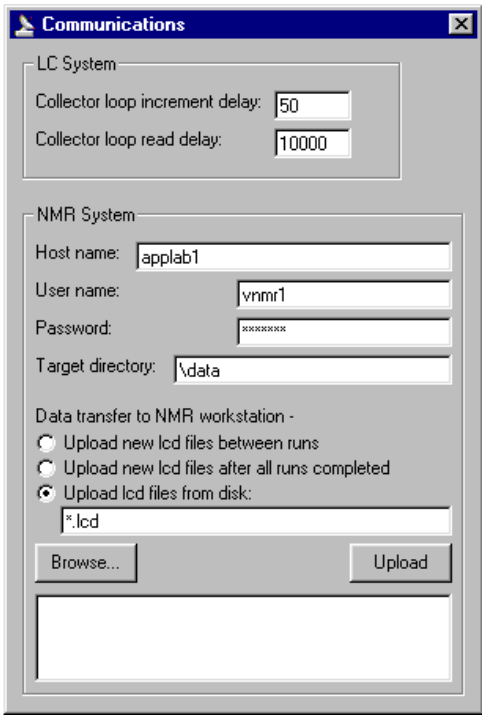


Figure 134. Communications Window

Analyte Collector Window

The Analyte Collector window, shown in Figure 135, is the software interface to the analyte collector option. The window duplicates the ability to switch the LC bypass valves as well as stepping, homing, and moving the analyte collector valve to a specific loop.

The window includes the following fields and buttons:

- Collector Status field displays the collector valve and its current position.
- Initialize button moves the value to position 1.
- Step button increments the valve position by 1
- Bypass button moves the valve to position 16.

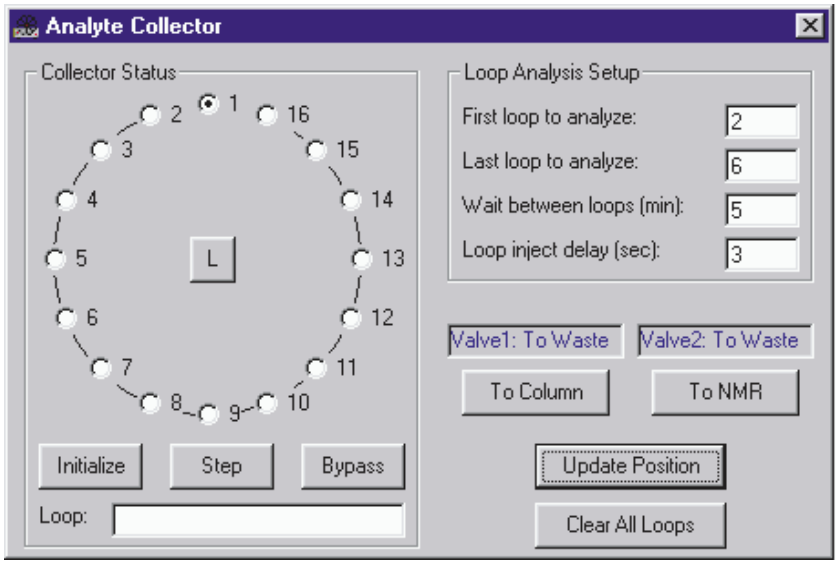


Figure 135. Analyte Collector Window

- Update Position button synchronizes the analyte collector window to the actual reading on the analyte collector.

Initializing the Analyte Collector

When Cascade is first set up on a new system or the analyte collector option is first installed, the program must be synchronized with the positions of the bypass valves and the analyte collector loop position.

To synchronize Cascade with the hardware, make sure that the analyte collector on-line option has been selected in the advanced options sections of the System Configuration window. Then click the Column and NMR probe bypass buttons two or three times. After the second click, the bypass valve indicators should show the correct status.

The loop collector valve probably displays a different position on the module box than shown by Cascade. To synchronize the positions, click on the Update Position button.

Analyte Collection

To store separated peaks in an LC chromatogram in individual loops, use the analyte collection program.

1. Have the LC method activated in **System Control** in Star Chromatography.
2. In System Configuration, select **Analyte Collection**.
3. In the Monitor window, make sure that **Inhibit Apex Detection** is **not checked**.
4. Click on Start Run.

The analyte collection mode of operation, unlike other modes of operation, does not direct the effluent of the column through to the NMR probe. Analytes can be collected in an environment completely removed from the NMR spectrometer and then analyzed later.

The analyte collection modes uses apex detection to sense peaks in the chromatogram. Analyte collection is starting by directing the column output to loop 1. Once a peak is

detected, the delay timer is invoked. Upon expiration of the delay time, the 16-port valve is rotated to loop 2, thus trapping the first peak in loop 1. Effluent continues to pump through loop 2 until the next peak is sensed and the delay time is counted off. Sample 2 is then stored in loop 2 as the valve is rotated to loop 3.

Analyte Analysis

Once the samples have been collected in the loops, it is necessary to perform analyte analysis.

1. In the **Batch Scheduler**, select the name to save the analyte analysis data files.
2. In **System Configuration**:
    a. Select analyte analysis in the experiment type field.
    b. Enter the correct delay time as determined during calibrations.
    c. Set the run time to match the LC method.
    d. Make sure that wait for NMR complete is enabled.
3. Go to the **System Control page** in Star chromatography and activate an **LC method** designed for analyte analysis.

    This method does not perform injections and has a 1 or 2 minute run time on the pump and detector. (This assumes that the same method will be used to elute each loop. If different methods are needed, see the section on setting up an automation run in Star.)
4. Return to the analyte collector window and focus on the **Loop Analysis Setup Field**. Enter the first and last loop to be analyzed. Choose a wait between loops sufficient for the LC method to equilibrate. Leave the loop inject delay at 3 seconds to avoid detector rezeroing at the beginning of a loop run.
5. Go to the **Monitor Window** and check that **Inhibit Apex Detection** is **not checked**.
6. From the **Monitor Window**, click **Start Run**.

When the analyte analysis is started, Cascade moves the analyte collector to the loop position preceding the starting loop position. This position is required in order for the LC pump to come to equilibrium and give a ready signal. After the wait between loops has counted down, the run is started by the LC system and the loop injection delay is counted down. The collector is then switched to the starting loop. This cycle continues until all specified loops have been injected.

If you are present during analyte analysis, you can abort the loop wait if the pump and detector reach ready state before the wait between loops time has expired.

Chapter 14. LC-NMR Accessory Experiments

Sections in this chapter:

- 14.1 “LC1D—LC-NMR 1D Pulse Sequence,” this page
- 14.2 “WET1D—Water Eliminated through Transverse Gradients 1D,” page 457
- 14.3 “WETDQCOSY—WET Double-Quantum Filtered COSY,” page 458
- 14.4 “WETGCOS—WET PFG Absolute-Value COSY,” page 460
- 14.5 “WETGHMQCPS—WET PFG HMQC (Phase Sensitive),” page 462
- 14.6 “WETGHSQC—WET PFG HSQC, Absolute Value or Phase Sensitive,” page 463
- 14.7 “WETGMQCOSY—WET PFG Absolute-Value MQF COSY,” page 465
- 14.8 “WETNOESY—WET Nuclear Overhauser Effect Spectroscopy,” page 466
- 14.9 “WETPWXAL—WET Pulse Width X Channel Calibration,” page 469
- 14.10 “WETRELAYH—WET Relay-COSY, WET Double Relay-COSY,” page 471
- 14.11 “WETTNTOCS—WET TOCSY with Water Suppression,” page 474

14.1 LC1D—LC-NMR 1D Pulse Sequence

Applicability

LC1D is available on two-channel systems with the Varian PFG module.

Macro

The `lc1d` macro converts the current (S2PUL-style) experiment into an LC-NMR experiment suitable for the LC1D pulse sequence. Figure 62 shows a diagram of the LC1D pulse sequence.

Parameters

Standard parameters:

- `wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).
- `wetpwr` is the power level for the WET element.
- `pwwet` is the pw90 pulse width at `wetpwr`.
- `wetshape` is the pulse shape used during the WET element.
- `gtw` is the duration of the spoiling gradient (in seconds).
- `gzlvlw` is the amplitude of the `gtw` gradient.

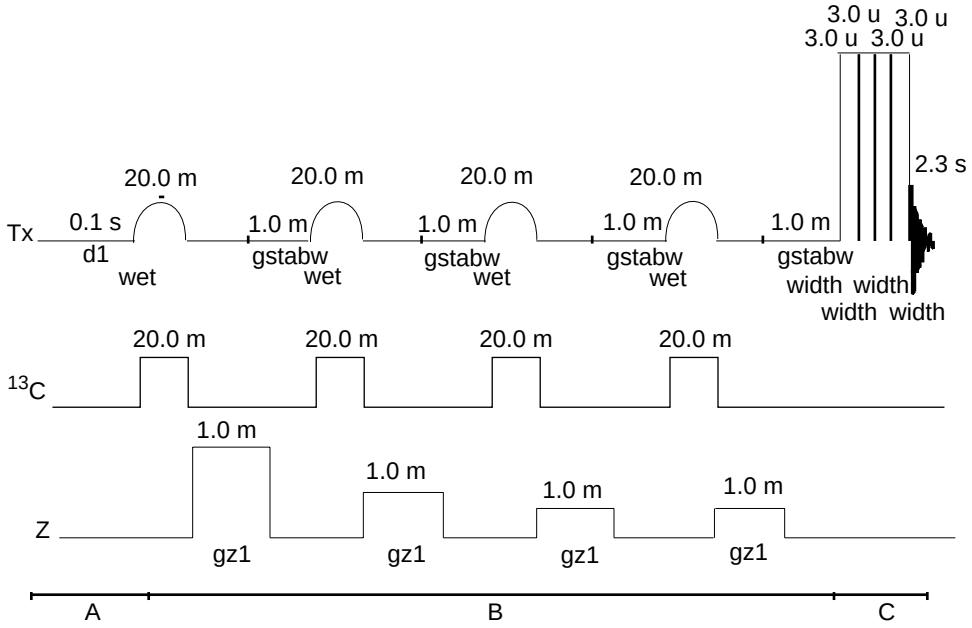


Figure 136. LC1D Pulse Sequence

- `gswet` is the recovery time following each gradient (try 0.002).
- `c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).
- `dofwet` is the ^{13}C decoupler offset used during WET.
- `dpwrwet` is the decoupler power level for `c13wet`.
- `dmfwet` is the `dmf` value used for `c13wet`.
- `dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).
- `composit` is a y/n flag determining whether a composite read pulse is used.

Additional (optional) parameters for automated LC-NMR:

- `inject` is a y/n flag controlling the line to the LC-NMR.
- `savefile` is a file name for automation.
- `nscans` is number of scans requested for LC-NMR (like `ni`).
- `curscan` is a counter.
- `ntrig` is the number of triggers from the LC to wait for (on the external gate line).
- `dtrig` is a trigger delay (in seconds).

Calibrations

Use the following steps to perform calibrations.

1. Enter `lc1d` to set up the `lc1d` pulse sequence. Next, using `composit='n'`, `tpwr=60` (approximately), and `wet='n'`, calibrate a 90° pulse. Using the LC solvent to do this will produce receiver/ADC overload, but set `gain=0` and vary the pulse around 360° and a calibration can be performed. Set pw to the value 90°.

2. Set `tpwr=tpwr-35` and obtain a normal spectrum. Place the cursor near the largest peak (assumed here to be CH_3CN) and enter `nl movetof` so that the largest peak is at zero. Enter `ai` to be sure you are in absolute intensity mode.
3. Set `wet='y'`, set the gradient parameters to reasonable values, set `wetshape='wet' wetpwr=-4 tpwr=tpwr+35`. Obtain a spectrum and verify that a significant degree of solvent suppression has occurred. Assuming it has, perform an array of `wetpwr` in steps of 0.5 to obtain an optimum value for best suppression.
4. Place the cursor on the next largest resonance (assumed to be HOD in this example) and enter `crl rfl=sw/2 nl:r1,slp` to capture the difference frequency between CH_3CN and HOD into the parameter slp. Enter `makeslpnames` to change `wetshape` into a name that contains the `slp` frequency. If there is no such shape in your `shapelib` yet, create it using `makeslpshapes` (or a series of frequencies at nearby frequencies).
5. Set `wetpwr` equal to the previous optimum+6 and obtain a new spectrum, which should show both CH_3CN and HOD suppressed. Set up an array of `wetpwr` again, this time in steps of 0.1, to obtain the optimum suppression.

14.2 WET1D—Water Eliminated through Transverse Gradients 1D

Applicability

WET1D is available on systems with the Varian PFG module.

Macro

The `wet1d` macro converts the current (S2PUL-style) experiment into an experiment which has wet parameters, but which is still suitable for the S2PUL pulse sequence.

Parameters

A full description of S2PUL, including a diagram of the sequence, is provided in the manual *Getting Started*.

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the gtw gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

14.3 WETDQCOSY—WET Double-Quantum Filtered COSY

Applicability

WETDQCOSY is available on systems with the Varian PFG module.

Macro

The `wetdqcosy` macro sets up parameters for a wet version of the DQCOSY pulse sequence, starting from either a WET1D or LC1D parameter set. Figure 63 is a diagram of the sequence.

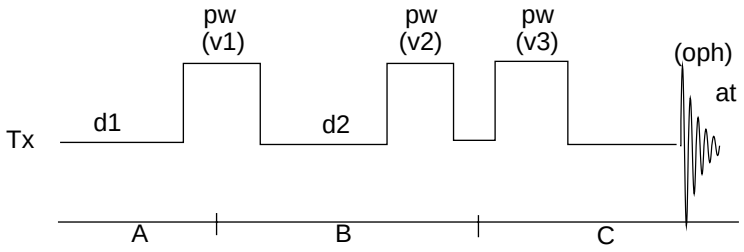


Figure 137. WETDQCOSY Pulse Sequence

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet is` the pw90 pulse width at `wetpwr`.

`wetshape` - the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`pw` is a 90° pulse on the observed nucleus.

`d1` is a relaxation delay (1 to 3 times the value of `t1`).

`d2` is the evolution time (`t1` period).

`at` is the acquisition time (`t2` period).

`sw` is the spectral width (usually `sw1=sw`, except for `phase=3`).

`ni` is the number of `t1` increments (set so that s`w1/ni=12`).

`phase=0` for 2D data in a `av` display (P-type peaks); `phase=1,2` for 2D hypercomplex data (States-Haberkorn method); `phase=3` for 2D TPPI data.

`sspul='y'` activates a homospoil–90–homospoil sequence that precedes d1; used to achieve a less oscillatory steady-state for 2D experiments where the recycle time is shorter than t_1.

`ss` is the number of steady-state transients; if `ss` is less than 0, `-ss` transients (with phase cycling of pulses and receiver) are performed before each experiment in either an arrayed or 2D experiment; if `ss` is greater than 0, ss transients are performed before only the first experiment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on `dm` but does depend on `dmm` and is activated as a part of `d1` if presat is greater than 0.

`nt` is a multiple of 8 (`phase=0`) (minimum); multiple of 4 (`phase=1`, `2`, or `3`) (minimum).

Phase Cycling

`v1` is the phase for first `pw` pulse.
`v2` is the phase for second `pw` pulse.
`v3` is the phase for third `pw` pulse.
`oph` is the phase for receiver.

```
v1  =  x  x  x  x  y  y  y  y  x  x  x  x  y  y  y  y
      -x -x -x -x -y -y -y -y -x -x -x -x -y -y -y -y
v2  =  x  x  x  x  y  y  y  y -x -x -x -x -y -y -y -y
v3  =  x  y -x -y  y -x -y  x
oph =  x -y -x  y  y  x -y -x -x  y  x -y -y -x  y  x
      -x  y  x -y -y -x  y  x  x -y -x  y  y  x -y -x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add 90∗ (`ix` – 1)° to `v1`, where `ix` is the increment counter. For `phase=0`, the subcycle of p-type peak selection is added in after the basic four-step cycle of `v3`.

Technique

To set up and acquire data, use the technique given in the description of Absolute-Value COSY, RELAY-COSY, or Double RELAY-COSY.

No phasing should be necessary for processing. For full processing, use menus or enter `wft2da`. For step-by-step processing, proceed as follows:

1. Enter `wft(1)` to Fourier transform the first increment of data. There should be no signals in the first increment of a WETDQCOSY.
2. Enter `wti` to start interactive weighting. Data from WETDQCOSY is phase-sensitive and usually processed using Gaussian weighting, which is the default weighting function. Adjust the Gaussian weighting so that the data decays to zero before the end of the window. Adjusting the weighting function on the first increment of the data sets the weighting function in the 2D time dimension t_2.
3. Enter `wft1da` to Fourier transform the t_2 dimension and display a contour map of f_2,t_1 showing individual interferograms. Click on the trace button and choose a trace through one of the interferograms. Enter `wti` to bring up interactive weighting of the interferogram. Adjust the weighting function as before.
4. Enter `wft2da` to complete the Fourier transformation.
5. Display and plot using the procedures given in the description of Absolute-Value COSY, RELAY-COSY, or Double RELAY-COSY.

Potential Problems

The WETDQCOSY experiment is much more sensitive to artifacts than the COSY experiment. WETDQCOSY experiments are not symmetrized so `t1` noise is a much greater problem. To minimize `t1` noise, make sure that the 90-degree pulse is correct before beginning the experiment. Another problem that can arise is the presence of artifacts due to pulsing too rapidly. Make sure you set `d1` to at least 1 to 3 times the T_1 value of the protons in the sample. Run the sample nonspinning and use homospoil pulses.

References

Smallcombe, S. H.; Patt, S. L.; Keifer, P. A. *J. Magn. Reson.* **1995**, *117* (Series A), 295–303.

Piatini, U.; Sørenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc*. **1982**, *104*, 6800-6801.

Rance, M., et al *Biochem. Biophys. Res. Comm*. **1983**, *117*, 479–485..

14.4 WETGCOS—WET PFG Absolute-Value COSY

Applicability

WETGCOSY is available on systems with the Varian PFG module.

Macro

The `wetgcosy` macro sets up parameters for the pulse sequence WETGCOSY (WET Pulsed Field Gradient Absolute-Value Correlated Spectroscopy), starting from either a WET1D or an LC1D parameter set. Figure 64 shows the WETGCOSY pulse sequence diagram.

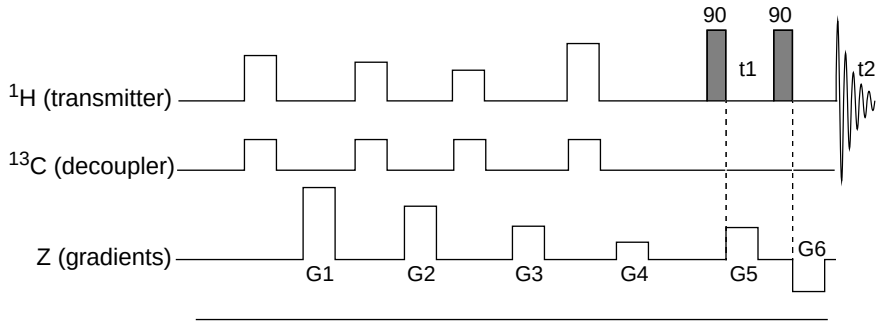


Figure 138. WETGCOSY Pulse Sequence

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a `y/n` flag determining whether a composite read pulse is used.

`gzlvl1` is the gradient amplitude (–32768 to +32768; use 3000).

`gt1` is the gradient duration (in seconds, 0.002).

`grise` is the gradient rise and fall time (in seconds, 0.00001).

`qlvl` is 1 (quantum selection level).

`gstab` is an optional delay for stability (in seconds).

`phase=1` selects echo N-type coherence selection (this is the default),
`phase=2` selects anti-echo P-type coherence selection.

Processing

Process N-type data with `wft2d(1,0,0,1)`; process P-type data with `wft2d(1,0,0,-1)`. The `'t2dc'` argument to `wft2d` may be useful.

14.5 WETGHMQCPS—WET PFG HMQC (Phase Sensitive)

Applicability

WETGHMQCPS is available on two-channel systems with the Varian PFG module.

Macro

The `wethmqcps` macro sets up parameters for the wet version of the pulse sequence GHMQCPS (Pulsed Field Gradient Heteronuclear Multiple-Quantum Correlation, phase-sensitive), starting from either a WET1D or an LC1D parameter set.

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`j` is `1JXH` in Hz (140 typical for ^{1}H-^{13}C).

`pwxlvl` is the decoupler pulse power level.

`pwx` is the decoupler pulsed `pw90`.

`gzlvl1` is the gradient amplitude (-32768 to +32768).

`gt1` is the gradient duration (in seconds, 0.001).

`gzlvl2` is the gradient amplitude (-32768 to +32768).

`gt2` is the gradient duration (in seconds, 0.001).

`gzlvl3` is the gradient amplitude (-32768 to +32768).

`gt3` is the gradient duration (in seconds, 0.001).

`grise` is the gradient rise and fall time (in seconds; 0.00001).

`gstab` is the optional delay for stability (in seconds).

`gzlvl1, gzlvl2`, and `gzlvl3` and their times (`gt1, gt2`, and `gt3`) may eventually be fixed in their relationship (i.e., 2:2:-1, 0:4:-3, etc.).

For ^{13}C, try `gzlvl1=20000 gt1=0.002 gzlvl2=20000 gt2=0.002 gzlvl3=-10050 gt3=0.002` and array `gzlvl3` for maximum signal.

Processing

If `gzlvl3` is the same sign as `gzlvl1` and `gzlvl2` (N-type data), process with `wft2d('t2dc')`.

If `gzlvl3` is opposite in sign to `gzlvl1` and `gzlvl2` (P-type data), process with `wft2d('t2dc','ptype')`. The sequence sets three gradients, all separately.

14.6 WETGHSQC—WET PFG HSQC, Absolute Value or Phase Sensitive

Applicability

WETGHSQC is available on two-channel systems with the Varian PFG module.

Macro

The `wetghsqc<(nucleus)>` macro sets up parameters for a WET version of the pulse sequence GHSQC (Pulsed Field Gradient Heteronuclear Single-Quantum Correlation), absolute value or phase sensitive, starting from either a WET1D or an LC1D parameter set. The optional argument `nucleus` is ^{13}C or ^{15}N, for example, `ghsqc(15N)`. The default value of `nucleus` is ^{13}C.

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`gt1` is the first gradient duration.

`gzlvl1` is the first gradient power level.

`gt2` is the second gradient duration.

`gzlvl2` is the second gradient duration.

The gradients need to maintain a 4:1 (^{13}C) or 10:1 (^{15}N) ratio. The ratio can be controlled with either amplitude or time.

`pwxlvl` is the decoupler power level for hard decoupler pulses.

`pwx` is the pulse width for hard decoupler pulses.

`dpwr` is the power level for decoupling.

`dmf` is controlled by `dpwr`.

`j` is a one-bond heteronuclear coupling constant (140 for ^{13}C, 90 for ^{15}N).

`bigt` is a constant time for X evolution (try 3 ms).

`satmode` is 'y' for transmitter presaturation.

`satdly` is the presaturation delay used if `satmode='y'`.

`satpwr` is the presaturation power.

`satfrq` is the frequency desired for presaturation.

`f1180` is a y/n flag for 1/2 dwell starting `t1` evolution delay.

`nt` works with `nt=1` but `nt=2` improves data.

Use `phase=1,2` to select N,P-type selection (to be sorted later); use `phase=1` to generate an absolute-value data set.

- For ^{13}C try `gzlvl1=20000 gt1=0.002 gzlvl2=-19850 gt2=0.0005`.
- For ^{15}N try `gzlvl1=30000 gt1=0.0025 gzlvl2=-14850 gt2=0.0005`.

Processing

Use `wft2d('t2dc',1,0,0,1,0,1,1,0)` for `phase = 1,2` (phase up first increment, then `rp=rp-45`).

Use `wft2d(1,0,0,1)` for `phase=1`.

14.7 WETGMQCOSY—WET PFG Absolute-Value MQF COSY

Applicability

WETGMQCOSY is available on systems with the Varian PFG module.

Macro

The `wetgmqcosy` macro sets up parameters for a wet version of the pulse sequence GMQCOSY (Pulsed Field Gradient Multiple-Quantum Filtered Correlated Spectroscopy), starting from either a WET1D or an LC1D parameter set.

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`gzlvl1` is the gradient amplitude (use 10000 since it is multiplied by `qlvl+1`).

`gt1` is the gradient duration (in seconds, 0.001).

`grise` is the gradient rise and fall time (in seconds, 0.00001).

`qlvl` is 2 (quantum selection level for DQF COSY).

`gstab` is an optional delay for stability (in seconds).

`phase=1` selects echo N-type coherence selection (this is the default),
`phase=2` selects anti-echo P-type coherence selection.

Gradient levels for organic samples: try `gzlvl1 = 10000 gt1 = 0.003`

Gradient levels for H_2O samples: try `gzlvl1 = 10000 gt1 = 0.008`

Processing

Process N-type data with `wft2d(1,0,0,1)`; process P-type data with `wft2d(1,0,0,-1)`. The `'t2dc'` argument to `wft2d` may be useful.

14.8 WETNOESY—WET Nuclear Overhauser Effect Spectroscopy

Applicability

WETNOESY is available on systems the Varian PFG module.

Macro

The `wetnoesy` macro sets up parameters for a wet version of the NOESY laboratory frame Overhauser or 2D exchange pulse sequence, starting from a WET1D or an LC1D parameter set. It can be performed in phase-sensitive or absolute value mode. Either TPPI or the hypercomplex method can be used to achieve f1 quadrature in a phase-sensitive presentation. No attempt is made to suppress J-cross peaks. Figure 65 shows the sequence.

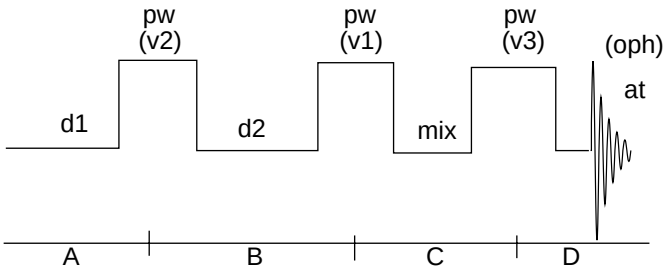


Figure 139. NOESY Pulse Sequence

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`). It is located at the end of the mix time.

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`pw` is a 90° pulse on the observed nucleus (`power=tpwr`).

`d1` is the relaxation delay (1 to 3 times the value of T_1).

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (`sw1=sw` usually, except for `phase=3`).

`ni` is the number of t_1 increments (set up by default so that `sw1/ni=12`).

`mix` is the mixing time for magnetization exchange. The wet element is located at the end of `mix`; hence, mix time must be sufficiently long to allow the wet element to occur, or an error message results.

`phase=1,2` is the suggested value; `phase=0` for P-type peak selection for `av` display; `phase=1,2` for 2D hypercomplex data (States-Haberkorn method); `phase=3` for 2D TPPI data.

`sspul='y'` activates a homospoil-90-homospoil sequence prior to `d1`, which achieves a less oscillatory steady-state in 2D experiments where recycle time is less than T_1.

`ss` is the number of steady-state transients; if `ss` is less than 0, `–ss` transients (with phase cycling of pulses and receiver) are performed before each experiment in either an arrayed or 2D experiment; if `ss` is greater than 0, `ss` transients are performed before only the first experiment.

`presat` is decoupler presaturation period using a decoupler power specified by `dhp` or `dpwr`. `presat` does not depend on dm but does depend on dmm and is activated as a part of `d1` if `presat` is greater than 0.

`dm='nnnn'` is no decoupler presaturation (unless `presat` is greater than zero); `dm='nynn'` is decoupler presaturation during evolution period; `dm='nnyn'` is decoupler presaturation during mixing period; `dm='nnny'` is homonuclear decoupling during t2; `dm='nyyn'` is recommended when solvent saturation is needed.

`nt` is a multiple of 8 (minimum) for `phase=1,2` or `phase=3`; `nt` is a multiple of 16 (minimum) for `phase=0`.

Phase Cycling

`v1` is the phase for the second `pw` pulse.
`v2` is the phase for the first `pw` pulse.
`v3` is the phase for the third `pw` pulse.
`oph` is the phase for receiver.

```
v1  =  x  x  x  x  x  x  x  x  y  y  y  y  y  y  y  y
      -x -x -x -x -x -x -x -x -y -y -y -y -y -y -y -y
v2  =  x -x  x -x  x -x  x -x  y -y  y -y  y -y  y -y
v3  =  x  x  y  y -x -x -y -y  y  y -x -x -y -y  x  x
oph =  x -x  y -y -x  x -y  y  y -y -x  x -y  y  x -x
      –x  x -y  y  x -x  y -y -y  y  x -x  y -y -x  x
```

These phases are for `phase=1`. For `phase=2`, add 90° to `v1`. For `phase=3`, add `90*(ix – 1)°` to `v1`, where `ix` is the increment counter. For `phase=0`, the P-type selection subcycle is added in after the first eight steps in the phase cycle.

For `phase=3`, remember that `sw1` equals `2*sw` (for homonuclear experiments) or that `sw1` must be set to twice the desired value (for heteronuclear experiments).

If `mix` is arrayed, then `phase=3` is necessary in order to be able to process the data with the `wft2dac` macro.

Technique

Because WETNOESY is a phase-sensitive 2D experiment, WETNOESY spectra may need to be phased before good results are obtained.

1. A good sample to try the first time would be a 5% sucrose sample in D_2O. This sample is concentrated enough that good data can be obtained in a relatively short time. If not there already, join experiment 1 by entering `jexp1` or by using menu buttons. Set up a proton in D_2O by clicking on the menu buttons Setup, H1, D2O. Acquire a spectrum.
2. Narrow the spectral window to include only the peaks of interest by placing a cursors about 1 ppm upfield and 1 ppm downfield of the region containing the peaks and enter `movesw`. This greatly decreases the amount of spectrum that is just noise and provides better digital resolution in the spectrum and decreased experiment time. Generally you want to obtain better digital resolution in phase-sensitive experiments than in absolute-value experiments.
3. Enter `go` to acquire the spectrum with the new window and phase the data. This spectrum will serve as a reference spectrum for the WETNOESY.
4. Enter `mp(1,4) jexp4` to move the parameters from experiment 1 to experiment 4 and join experiment 4.
5. Enter `noesy` to set up the WETNOESY experiment and display an estimate of the time required to perform the experiment.
6. If you are running the sucrose sample, set `nt=16`, `ni=128`, `np=1024`, `fn=1024`, `fn2=1024`, and `d1=2`. The experiment takes about three hours.
7. Enter `go` to acquire the data.
8. Enter `wft(1)` to Fourier transform the first increment of data. The spectrum appears inverted, but this is normal. When the spectra are Fourier transformed, the diagonal is below the plane of the 2D, and NOE crosspeaks due to positive NOEs appear positive, and crosspeaks due to negative NOEs are negative. Phase the spectrum, leaving it properly phased but inverted. This sets the phase for the F_2 dimension of the 2D.
9. Enter `wti` to start interactive weighting. WETNOESY data is phase-sensitive and usually processed using Gaussian weighting, the default weighting function calculated by the setup macro. Adjust the Gaussian weighting so that data decays to zero before the end of the window. Adjusting the weighting function on the first increment of the data sets the weighting function in the 2D time dimension t_2.
10. Enter `wft1da` to Fourier transform the t_2 dimension. A contour map of F_2,t_1 is displayed, showing individual interferograms. Click on trace and choose a trace through one of the interferograms. Enter `wti` to bring up interactive weighting of the interferogram. Adjust the weighting function as before.

11. Enter `wft2da` to complete the Fourier transformation.
12. The F_1 dimension may now need phasing. To phase F_1, click on trace and select a trace at the top (upfield) section of the 2D. Enter `ds` to display the trace, and phase normally using the parameter `rp`.
13. Enter `dconi` to redisplay the contour map with a new `rp`. Click on trace again, and select a trace at the bottom (downfield) section of the 2D. Enter `ds` and click on phase. Move the cursor upfield and click. *Do not adjust the phase at this point.* Clicking at this point sets `rp` and retains the `rp` value obtained previously. Move downfield and click. Adjust phase normally. This adjusts `lp`. Enter `dconi` to redisplay the properly phased 2D.
14. Enter `plcosy` to plot the data. The `plcosy` macro is general and plots all homonuclear correlated data.

Potential Problems

Unlike the COSY experiment, obtaining good NOESY spectra requires proper values of 90° pulse width and a consideration of delay times. Make sure that the 90° pulse is correct before beginning the experiment. If 90° pulse is incorrect, many “COSY type” (i.e. antiphase cross-peaks) appear, complicating the analysis. In small molecules, some “COSY-type” cross-peaks may be unavoidable even when everything is carefully calibrated. Fortunately these can be easily distinguished because of their antiphase nature, i.e. the cross-peaks have both positive and negative components but true NOE peaks are pure absorptive.

Another possible problem is the presence of artifacts due to pulsing too rapidly. Make sure you set `d1` to at least 1 to 3 times the T_1 of the protons in the sample. The NOESY experiment must be interpreted more carefully than the COSY experiment because cross-pulses arise from COSY interaction as well as dipolar interaction.

Reference

Smallcombe, S. H.; Patt, S. L.; Keifer, P. A. *J. Magn. Reson.* **1995**, *117* (Series A), 295-303.

States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

14.9 WETPWXAL—WET Pulse Width X Channel Calibration

Applicability

WETPWXCAL is available on two-channel systems with the Varian PFG module.

Macro

The `wetpwxcal` macro sets up parameters for the WETPWXCAL pulse sequence, starting from a WET1D or an LC1D parameter set. WETPWXCAL is used to calibrate the pulse width characteristics of the probe’s decoupler channel(s) in triple resonance and indirect detection LC-NMR experiments. WETPWXCAL can also be used to determine the rf field homogeneity of the decoupler. This calibration is a more sensitive measure of the decoupler X pulse widths than the first increment of HMQC. WETPWXCAL is designed for dual-broadband systems only and does not support “reverse mode” acquisition.

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in seconds).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet` —hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`pwx1` is the proton pulse width on first decoupler (in µs).

`pwx2` is the proton pulse width on second decoupler (in µs).

`pwx3` is the proton pulse width on third decoupler (in µs).

`jC13` is the ^{13}C-^{1}H coupling constant.

`jN15` is the ^{15}N-^{1}H coupling constant.

`jP31` is the ^{31}P-^{1}H coupling constant.

`jname` is the selected calibration nucleus.

Technique

1. Starting from either a WET1D or an LC1D parameter set, enter `wetpwxcal` and answer the prompts:

   ```
   Use decoupler 1, 2, or 3 [1]
   ```

   and

   ```
   Calibrate C13, N15, or P31 [C13]
   ```

   Pressing Return as an answer selects the default response enclosed in square brackets.

2. Array the parameter `pwx1`, starting at 0, making sure the delay `d1` is reasonably long compared to the ^{1}H relaxation times.

3. Phase the first spectrum (`pwx1=0`). All peaks null when `pwx1` is a 90° pulse.

4. If a second decoupler is present, array `pwx2` to calibrate the 90° pulse width on that decoupler. If a third decoupler is present, array `pwx3` to calibrate the 90° pulse width on that decoupler.

14.10 WETRELAYH—WET Relay-COSY, WET Double Relay-COSY

Applicability

WETRELAYH is available on systems with the Varian PFG module.

Macro

The `wetcosy` macro sets up the WETCOSY parameters, starting from a WET1D or an LC1D parameter set. The `relayh` macro sets up parameters for absolute-value COSY, single RELAY-COSY, or double RELAY-COSY pulse sequences. Figure 66 is a diagram of the absolute-value COSY sequence, and Figure 67 is a diagram of RELAY-COSY, single and double.

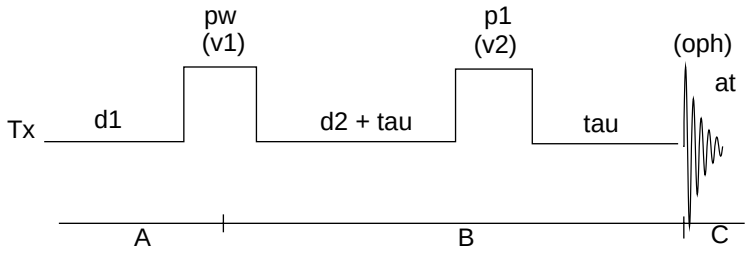


Figure 140. Absolute-Value COSY Pulse Sequence

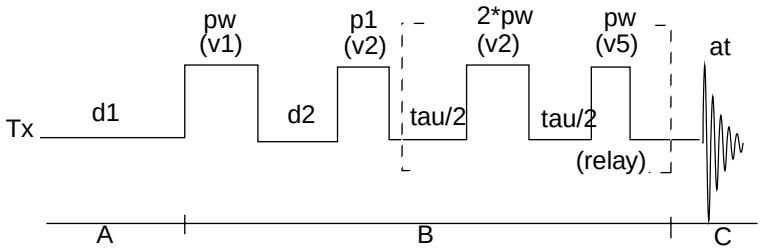


Figure 141. RELAY- COSY Pulse Sequences

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in sec).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`relay` is the number of relays to be performed:
if `relay=0`, a normal absolute-value WETCOSY is performed.
if `relay=1`, a WET RELAY-COSY is performed.
if `relay=2`, a WET DOUBLE-RELAY-COSY is performed.

`pw` is a 90° pulse.

`p1` is a 90° pulse if relay is not equal to 0; if relay is equal to 0, diagonal peaks can be deemphasized by using pulses greater than 90° when doing P-type peak selection or by using pulses less than 90° when doing N-type peak selection.

`d1` is a delay set to 1 to 3 times T_1.

`d2` is the evolution time (t_1 period).

`at` is the acquisition time (t_2 period).

`sw` is the spectral width (`sw1=sw`).

`v1`, `v2`, and `v5` are pulse phases. oph denotes the phase of the receiver.

`ni` is the number of t_1 increments (set so that `sw1/ni=6` by default). If speed is essential, `ni` can be decreased.

`tau` is the propagation time for long-range COSY (`relay=0`) and for relayed COSY (`relay` greater than 0).

Technique

The WETCOSY experiment, as implemented on UNITY*INOVA*, UNITY*plus*, and UNITY systems, can be run several ways. The actual experiment macro called is `wetcosy`. If the parameter `relay` is set to 0 (the default), a normal WETCOSY is performed.

The standard setup uses two 90° pulses and is the traditional way to run COSY experiments. The second pulse can be changed to a 45° pulse to decrease the size of the diagonal. This technique is called COSY-45. Besides decreasing the diagonal, COSY-45 emphasizes active coupling partners relative to passive couplings so if sufficient digital resolution is present, the crosspeaks may show an interesting multiplet structure. To emphasize long-range couplings, increase the setting of the parameter `tau` to 0.2 seconds.

To Set Up and Acquire:

1. Set up by acquiring a 1D proton experiment in `exp`*n*, where *n* is 1, 2, 3, etc.
2. Narrow the ^{1}H spectral window to leave approximately 1 ppm on either side of the peaks of interest by using two cursors and entering `movesw`.
3. Reacquire the ^{1}H with the new spectral window.

4. Phase the ^{1}H spectra.
5. Move the FID to another experiment (e.g., to move an FID in `exp1` to `exp2`, `enter mf(1,2))`.
6. Enter `wetcosy` to modify the parameters for the WETCOSY experiment. The `wetcosy` macro calculates appropriate weighting functions.
7. Turn the spinner off.
8. Enter `au` to acquire data.

To Fourier Transform:

1. Enter `wft2d`. This performs weighted Fourier transforms in both dimensions and displays the data as a contour map.
2. Adjust threshold and vertical scale.

To Adjust Weighting During Processing:

1. Enter `wft(1)` to Fourier transform the first increment of data.
2. Enter `wti` to start interactive weighting.
3. Absolute-value data is usually processed using sinebell weighting. This is the default weighting function calculated by the macro setting up the experiment. Adjust the sinebell so that data decays to zero before the end of the window. Adjusting the weighting function on the first increment of data sets the weighting function in the 2D time dimension t_2.
4. Enter `wft1d` to Fourier transform the t_2 dimension. A contour map of f_2,t_1 is displayed, showing the individual interferograms.
5. Click on the trace button and choose a trace through one of the horizontal interferograms.
6. Enter `wti` to bring up interactive weighting of the interferogram. Adjust the weighting function the same as before.
7. Enter `wft2d` to complete the Fourier transformation.
8. Enter `foldt` to symmetrize the data.
9. You can adjust the vertical scale and threshold to provide a better display in which the cross peaks and diagonal are more easily seen. Adjust the vertical scale by changing the value of the parameter `vs` (e.g., `vs=12)`.

   If the vertical scale is too high, you can decrease it by entering `vs=vs/10` (or a similar divisor) to drop the vertical scale by that amount. Adjust the threshold by using the mouse to adjust the sliding color scale beside the 2D contour plot.
10. Enter `dcon` to display a non-interactive color intensity map, or enter `dconi` to permit interaction.
11. Enter `dpcon(12,1.3)` to see what your data will look like if plotted with 12 contours spaced 1.3 levels apart. If you wish, try other arguments for `dpcon` to see how other values of contours and levels will look when plotted.
12. Enter `plcosy(8,1.5)` to plot the COSY or RELAYH data with eight contours spaced 1.5 levels apart. Try other values if you want.

The macro `plcosy` actually takes three arguments: the number of contours, the spacing of levels, and the experiment number that contains the 1D data. If there is no third argument, `plcosy` assumes that the 1D data is in experiment 1. Therefore, `plcosy(8,1.5)` plots the contour, retrieves the 1D data, and plots it above the contour.

13. Enter `dconi` to make an expansion of the data and redisplay the contour map if it is not present. Expand around the upfield region by using the left mouse button to determine the lower left corner and the right mouse button to determine the upper right corner, then click the expand button.
14. Click on the expand menu button.
15. Enter `plcosy` to plot this expanded region. Note that the appropriate 1D region is plotted on the top and to the side of the 2D plot.

Potential Problems

The WETCOSY experiment is very forgiving and usually works (to a greater or lesser extent) with a slightly incorrect 90° pulse width. The most common reason for failure of the experiment is that the value for `p1` is incorrect. Make sure that the 90° pulse is correct before beginning the experiment. Remember that `pw`, the second pulse, may be set to 45° to deemphasize the diagonal and provide a filter for passive couplings.

A second problem that can arise are artifacts caused by pulsing too rapidly. If you symmetrize the spectrum with `foldt` (normal in COSY), these should be minimized.

14.11 WETTNTOCS—WET TOCSY with Water Suppression

Applicability

WETTNTOCSY requires a T/R (transmit/receive) switch, as well as linear amplifiers and computer-controlled attenuators on the observe channel. WETTNTOCSY also requires the Varian PFG module.

Macro

The `wettntocsy` macro sets up parameters for a wet version of TOCSY (total correlation spectroscopy, also known as HOHAHA), starting from a WET1D or an LC1D parameter set. It features clean TOCSY (windowing) and MLEV16 + 60° spin lock (MLEV17). The transmitter must be positioned at the solvent frequency.

Parameters

`wet` is a y/n flag determining whether the WET element is used (which uses `pwwet`, `wetpwr`, `wetshape`, `gtw`, `gzlvlw`, and `gswet`).

`wetpwr` is the power level for the WET element.

`pwwet` is the `pw90` pulse width at `wetpwr`.

`wetshape` is the pulse shape used during the WET element.

`gtw` is the duration of the spoiling gradient (in sec).

`gzlvlw` is the amplitude of the `gtw` gradient.

`gswet` is the recovery time following each gradient (try 0.002).

`c13wet` is a y/n flag controlling the selective ^{13}C decoupling used during WET (which uses `dorwet`, `dpwrwet`, and `dmfwet`—hardcoded to be WALTZ).

`dofwet` is the ^{13}C decoupler offset used during WET.

`dpwrwet` is the decoupler power level for `c13wet`.

`dmfwet` is the `dmf` value used for `c13wet`.

`dz` is the delay following the wet element to allow longitudinal recovery (like a T1IR or WEFT inversion recovering through zero).

`composit` is a y/n flag determining whether a composite read pulse is used.

`pw` is a 90° pulse during `mlev` periods (at power level `tpwr`).

`p1` is a 90° excitation pulse (at power `p1lvl`).

`window` is a clean-TOCSY window (in microseconds).

`satdly` is the length of presaturation.

`satmode` is a flag for `presat` control:
`satmode='yn'` for during relaxation delay only,
`satmode='yy'` for during both “relaxation delay” and `d2` (recommended).

`phase=1,2` for hypercomplex phase-sensitive F_1 quadrature,
`phase=3` for TPPI phase-sensitive F_1 quadrature.

`sspul='y'` gives the `trim(x)-trim(y)` sequence at beginning of `d1` delay;
`sspul='n'` gives the normal `d1` delay.

`trim` is a spinlock trim pulse time (0.002 recommended).

`mix` is the mixing time (can be arrayed).

`nt` minimum is a multiple of 2, `nt` maximum is a multiple of 8 (recommended).

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Symbols

* notation in deconvolution, 208
+ mark by peak, 126
.Pbox_defaults file, 235
.Pbox_globals file, 235
[] (brackets) notation, 34

Numerics

10-mm sample tubes., 279
1D data processing
    LC-NMR, 431
1D experiments, 73
1D mode for LC-NMR data processing, 431
1D pulse sequences, 73
1D water suppression, 87
2D data processing for LC-NMR data, 431
2D Display Main Menu, 117, 123
2D Display Projection Menu, 117
2D DOSY experiment, processing, 55
2D DOSY sequences, 51
2D DOSY spectroscopy, 51
2D Exchange experiment, 155
2D FID matrices, 166
2D LC-NMR experiments, 425–427
    manual data acquisition, 426
    using enter program, 426
2D mode for LC-NMR data processing, 431
2D NMR, 89
2D Peak Picking menus
    Automatic, 123, 124, 127, 128
    Display, 124, 126, 129
    Edit, 123, 125, 127, 128
    Edit Secondary, 128
    File, 123, 126, 129
    Main, 123, 124
3D DOSY experiments, 58
    processing, 61
3D NMR, 129–134
4D data acquisition, 134
4-nucleus probes, 172
4-ply selective inversion pulses creation, 240
50-sample tray, 279
5-mm sample tubes, 279
9050 UV/Vis detector, 415

A

aa command, 297
Abort Acquisition button, 297
aborting
    acquisition in automation mode, 297
    gradient on flow experiment, 414
    LC-NMR experiment, 412
absolute-value color set, 115
Absolute-Value COSY pulse sequence, 138
absolute-value mode, 91, 109
absorption mode, 92
acetone in coolant bucket, 261
acosy command, 115
acosyold command, 115
Acqmeter display, 260
acqqueue directory, 309
acqstatus parameter, 264, 307
acquiring data on standard test samples
    VAST, 354
acquisition
    block processing, 306
    codes, 307
    macros, 301
    mode, 130
    order of events, 301
    status window, 93
acquisition computer
    state, 309
acquisitioninfo file, 309
add button, 217
add command, 214
add FID to add/subtract experiment, 214
Add/Sub button, 216
add/subtract buffer file, 213
add/subtract experiment, 213
Add/Subtract Menu, 213
addi command, 216
addition and subtraction of data, 213
additive weighting constant, 97
addpar macro, 90, 105, 106, 123, 134
adept command, 81
adiabatic decoupling, 241
adiabatic rf sweep waveform creation, 230
Adjust button, 125, 125, 128
air conditioning cycling, 264
Air valve, 360
alfa parameter, 99
amplifier lights on PFG amplifiers, 373
amplifier operation for PFG, 373
amplifiers used with indirect detection, 173
amplitude modulated data, 104
analyte
    analysis, 428, 453
    collection, 427
    collection program, 452
    collector operation, 427–429
    concentration, 410
Analyte Collector, 427
Analyte Collector window, 451
analytes, 414
analyze command, 46, 220
analyze.inp file, 45
analyze.list file, 220
analyze.out file, 220, 221
angled brackets (<>) notation, 30
apt macro, 74
APT pulse sequence, 74
aptaph macro, 75
Arm Z scale (mm), 361
aromatic solvents in coolant bucket, 261
array for a parameter, 32
array macro, 34
array of 1D spectra, VAST, 323
array of data, 32
array parameter, 34, 133
arraydim parameter, 36, 215
arrayed 1D experiment, 89
arrayed 2D and 3D, 92
arrayed 3D data set, 107

arrayed temperature operation, 265
arrayed VT experiments, 262
arraying order and precedence, 34
asize macro, 109
ASM-100 sample changer, 267
assign macro, 203, 206
assign nearest calculated transitions, 206
Attached Proton Test, 74
attn parameter, 238
au macro, 299, 302
auto assign button, 206
Auto button, 124
auto command, 295
auto_au macro, 296, 300
autodept macro, 81
autodir variable, 296
autogo
    command, 295, 296
    VAST automation, 315
autoinfo file, 308
autolocking
    enhancements, 303
Automake Shimmap button, 381
automated shimming, 383
Automated Shimming menu, 389, 392
automatic
    2D peak picking, 120, 127
    analysis of COSY data set, 115
automatic gain, 35
automatic sample changer accessory, 267
automatic teller machine (ATM) cards caution, 27
automation
    files that control VAST operation, 363
    VAST, 315
automation acquisition codes, 307
automation directory, 294, 307
automation file, 295
automation mode, 267, 296
automation run, 267
    actions when finished, 297
    adding samples during progress, 292
    entering samples, 288, 295
    experiment files, 295
    interacting with acquisition, 297
    multiple users, 288, 295
    parameters, 297
    starting, 295
    starting a run, 295
autoname parameter, 297, 308
autoscale macro, 38, 221
autoscaling, 38
Autoshim on Z button, 378
autoshimming, enhancements, 303
av1 command, 109
awc parameter, 97
awc1 parameter, 97
awc2 parameter, 97
axial artifacts, 181
axis labeling, 133
axis parameter, 111

B

back-flushing runs, 414
background operation, 131
Backup button, 129
Backup File button, 126
backwards linear prediction, 108
band broadening, 410
baseline correction, 55, 100, 109
baseline spikes, 260
Batch Scheduler window, 450
bc command, 100, 109
bearing air supply, 263
bilinear rotation decoupling, 179
binary peak file, 121, 121, 126, 129
binom macro, 75
BINOM pulse sequence, 75
binomial water suppression, 75
biopolymer, 166
bipolar pulse pair stimulated echo experiment, DOSY, 52
bipolar pulse pair stimulated echo INEPT experiment, DOSY, 54
BIRD pulse nulling effect, 179
Bloch equation calculations, 226
Bloch Simulation subwindow, 228
Bloch simulator, 232, 238
boresize parameter, 375
Both button, 124
Box button, 117, 124, 125, 217
brackets ([]) notation, 34
broadband probes, 172
Bruker FID data, 105
bs parameter, 35
bscor parameter, 238
bsim parameter, 238
bw parameter, 239

C

c13p macro, 302
C13WET
    LC-NMR, 406
    VAST, 319
calculate
    simulated spectrum, 205
    theoretical spectrum, 201
calculated transitions file, 205
calculated transitions intensity, 205
calculating
    field maps, 381
calfa macro, 99
calibrate
    flow rate parameters, VAST, 351
    probe volume, control data set, VAST, 346
    probe volume, control verification using liquid handler, 347
    probe volume, finding optimum value, 349
    probe volume, VAST, 345
    pulse width, 37
    sample temperature curve, 262
    sample volume, VAST, 350
    volumes and flow rates, VAST, 345–354
    XYZ coordinates of the rinse station, 354
calibrations for carbon indirect detection, 187
calibrations for nitrogen indirect detection, 199
Calibrations pane, gilson window, 355, 361

Cancel button, 117, 119
cancellation efficiency, 181
Carousel autosampler, 267
  adjusting eject air, 272
  adjusting sample float height, 272
  error codes, 277
  loading and unloading samples, 273
  locked mode, 270
  mounting the carousel, 271
  removing the carousel, 271
  run mode, 270
  using the carousel, 268
Carr-Purcell Meiboom-Gill T2, 76
carryover
  evaluating, 355
Cascade LC program, 401
Cascade LC Stop Flow Monitor program, 443
Cascade monitor window, 445
Cascade program, 443
cautions defined, 25
cdc command, 100
center command, 109
centersw macro, 114
centersw1 macro, 114
centersw2 macro, 129
cexp macro, 31
cfpmult macro, 100
change bar, 30
change samples
  automatically from the LCNMR/STARS pane, VAST, 315
  manually, VAST, 314
  using the enter program, VAST, 314
  VAST, 314
chemical shift analysis commands, 224
chemical shifts, 204
chlorinated solvents in coolant bucket, 261
chromatograms, typical, 441
chromatography, 435
CJ compensator, 264
cla macro, 205
cla parameter, 204
clamp parameter, 205
class C amplifier systems, 173
cleanexp macro, 302
clear
  add/subtract buffer, 214
  file of line assignments for iteration, 205
Clear button, 125
clfreq parameter, 204
clindex parameter, 205
clradd command, 214
coef file, 133
cold junction (CJ) compensator, 264
color intensity and CombiPlate, 333, 335
color intensity map, 112
color/grayscale adjustment bar, 115
combine all peaks within an area, 125
Combine button, 125
combine peaks, 122
CombiPlate
  analyzing data, 336
  and integral region colors, 333
  data analysis and VNMR, 332
  data for analysis without VNMR, 334
  fixing the color map, 336
  preparing VNMR data for analysis with, 331
  using for data analysis, 331–337
combiplate, command in UNIX window, 337
combishow, 332, 337
commands
  chemical shift analysis, 224
  DOSY, 56
comment assigned to peak, 122
Comment button, 126, 128
common coefficients for wft2d processing, 104
common solvents, typical flow rates for, 353
completed transients, 93
complex FT processing, 105
complex interferograms, 101
composite pulse creation, 230
configure VNMR for the VAST accessory, 344
contour plot, 112
control data set for probe volume calibration, 346
control temperature, 263
conventions used in manual, 29
convertbru command, 105
coolant bucket, 261, 263
copying current binary peak file, 126
correct spectral drift, 101
corrected-difference spectroscopy, 212
Correlated Spectroscopy, 141
correlation analysis, 218
COSY
  acquisition procedure, 91
  data automatic analysis, 115
  data symmetrization, 114
  pulse sequence, 141
COSY experiment, 139
  phasing, 97
cosy macro, 91, 141
cosyps macro, 141
COSYPS pulse sequence, 141
coupling constants, 204
cpmgt2 macro, 76
CPMGT2 pulse sequence, 76
cptmp macro, 302
cpx macro, 232, 242
cr parameter, 114
cr1 parameter, 114
cr2 parameter, 129
create
  2D parameters, 90
  2D peak picking parameters, 123
  3D coefficient file, 133
  3D parameters, 106, 130
  4D parameters, 134
  experiment, 31
  shaped rf pulses, 230
  waveforms, 230
creating a pseudo 2D data set
  from a VAST data set, 323
  using vastglue, step by step example, 325
credit cards caution, 27
crl command, 114
crl1 command, 114
crl2 macro, 129
crof2 macro, 99

ct parameter, 35, 93
current experiment, 32
current FID number, 93
current transition assignments, 206
cursor
    move to center of spectrum, 114
    position, 114
Cursor button, 117, 124, 125, 217
custom display order, VAST data, 327
customizing macro operation, 301
cycled difference NOE experiment, 77
cyclenoe macro, 77

D

d0 parameter, 240
d1 parameter, 240
d2 parameter, 90, 92, 93, 129, 130, 132, 240
d2pul macro, 78
D2PUL pulse sequence, 78
d3 parameter, 106, 129, 130, 132, 132
d4 parameter, 134
da command, 33
da display, 90
dac_p2 parameter, 42
dac_x parameter, 44
daslp parameter, 106
data processing
    1D data from LC-NMR runs, 431
    2D data from LC-NMR runs, 431
    creating a pseudo 2D data set from an isocratic LC-NMR experiment, 430
    creating a pseudo 2D display form a VAST data set, 323
    FDM, 68
    glued VAST data sets, 325–327
    gradient on flow data, 414
    isocratic data, 430
    LC-NMR, 429–431
    NMRgram, 430
    Scout Scan data, 430
    VAST data sets, 323–327
data processing type, 93
data systems for indirect detection, 174
data tables, 92
Dbppste experiment, 52
Dbppsteinept experiment, 54
dc command, 99, 100
dc correction, 100
dc2d command, 101, 109
dcg parameter, 100
dcon command, 112
dconi command, 115, 132
dconi parameter, 116
dconn command, 112
dcyc parameter, 239
Deconvolution menu, 210
deconvolution of spectra into individual lines, 207
decoupler
    as transmitter, 78
    channel, 172
decoupling patterns creation, 230
decoupling power control, 187
defining integral regions, 43
delay time calibration, 431
delete
    all peak bounds, 122
    all peaks, 125
    experiment, 31
    peak bounds, 125
    peak nearest cursor, 122
    peak nearest the cursor, 125
    spectra from file, 38
delexp macro, 31
dels command, 38
delta2 parameter, 129
DEPT data processing, analyzing, plotting, 81
dept macro, 79
DEPT pulse sequence, 79
dept.out file, 81
deptgl macro, 81
DEPTGL pulse sequence, 81
deptproc macro, 82
deshapei macro, 243
detected spectral axis, 111
detection time, 90
df2d command, 113
dg, 90
dg2 macro, 107
dga command, 205
Dgcstecosy experiment (AV mode), 59
Dgcstehmqc experiment (AV mode), 60
DgcsteSL experiment, 53
dglp macro, 108
dgs macro, 260
diagonal peaks exclusion, 122
diagonally arrayed parameters, 34
diffusion
    coefficient, 45
    measurements, 40
    ordered spectroscopy, 48
    projection, showing, 58
digitization of lineshapes, 207
dispersion tails, 95
display
    3D plane, 134
    3D processing parameters, 107
    arrayed parameter values, 33
    connectivities, 115
    experiment library, 31
    exponential curves, 38
    file of line assignments for iteration, 205
    full 2D display, 119
    full screen, 109
    grid lines, 112
    image, 112
    line assignments file, 206
    next plane, 132
    non-whitewashed stacked spectra, 113
    peak bounds, 126
    peak label, 127
    peak marked with +, 126
    peak number, 126
    peaks on top of spectrum, 120
    polynomial curves, 38
    previous plane, 132
    projection of data on plane, 134
    second cursor, 118

shaped rf pulses, 225
simulated spectrum, 205
simulation parameter file, 205
spectra with whitewashing, 113
spectrum from array, 35
stacked 3D spectra, 113
subset of 3D planes, 132
theoretical spectrum, 209
trace at cursor position, 119
traces, 120
Display button, 124
display limits, 109
display mode parameter, 109
Display Shimmap button, 381
Distortionless Enhancement by Polarization Transfer, 79
dla macro, 205, 206
dlalong command, 206, 206
dll command, 206
dmg parameter, 109
dmg1 parameter, 109
dmg2 parameter, 129
doff parameter, 85
doneQ file, 295, 296, 297, 308, 308
Doneshot pulse sequence, Tcl-Tk acquistion panel, 52
DOSY experiments, 48–??
dosy macro, 56
DOSY tools, 49
dot1 command, 38
double PFG spin-echo, 400
Double RELAY-COSY pulse sequence, 138
Double-Quantum Filtered COSY, 143
Double-Quantum Transfer Experiment, 152
downfield peak shift, 106
dpcon command, 112
dpconn command, 112
DPFGSE pulse program, 244
DPFGSE pulse program listing, 244, 245
dplane macro, 132, 134
dprofile macro, 233, 243
dproj macro, 134
dpwr parameter, 187
dqcosy macro, 143
DQCOSY pulse sequence, 143
dres parameter, 237
drift correction, 134
drift correction calculation, 101
dry nitrogen gas, 260, 263
ds command, 35, 97, 108, 120, 240
ds2d command, 113
ds2dn command, 113
dshape macro, 258
dshapef macro, 232, 243
dshapei macro, 258
dsp command, 205, 209
dsplanes macro, 132
dss command, 36, 113
dssa command, 36
dssan command, 36
dssh command, 36
dsshn command, 36
dssl macro, 36, 113
dssn command, 36
dsvast, 326, 337
dsvast2d, 326, 338
dsww command, 113
dual-broadband system, 172
recabling for indirect detection, 185
dutycycle variable, 42

E

E-BURP-1 shaped pulse, 240
echo experiment, 43
Edit button, 124, 127
eject air adjustment, 272
elements
gradient stimulated echo, 49
energy level table, 205
enter command, 288, 294, 295, 296, 299, 299, 299
enter program
and custom stop-flow, 415
enter programming selections, 299
enter window
customizing, VAST, 362
enterQ file, 294, 296, 296, 297, 300, 308
error codes, 277
error messages, 282
error processing, 302, 306
ethylene glycol for VT calibration, 264
evaluating
carryover, 355
evolution time, 90
exchanger coil, 261
excitation sculpting, 400
exclude diagonal peaks, 122
exp1 command, 47
Expand button, 117, 119, 124, 217
expanded display, 119
experiment
library, 31
method, 294
parameter list, 300
queueing, 267
status on sample changer, 289
experiment 5 (exp5), 213
experimental frequency index of a transition, 205
experimental line listing limits, 206
experiments
2D DOSY, setting up, 51
2D LC-NMR experiments, 425–427
3-D DOSY, 58
3D-DOSY, setting up, 58
Dbppste, 52
Dbppsteinept, 54
Dgcstecosy (AV mode), 59
Dgcstehmqc (AV mode), 60
DgcsteSL, 53
DOSY, 48
LC-NMR, 410–427
oneshot DOSY, 54
on-flow LC, 410–414
stop-flow experiments, 414–420
time slice stop-flow LC-NMR, 421–425
expfit command, 220
expl command, 38, 219
expl command, 47

expl.out file, 221
expladd macro, 47
explib command, 31
Exponential Analysis Menu, 39
exponential curves analysis, 38
exponential signal change, 40
extract 2D planes from 3D data set, 132, 134

F

F1 Axial Displacement, 181
f_1 axis, 90
F1 phase detection, 181
F1, F2, and F3 dimensions, 131
f19p macro, 302
f1coef parameter, 133
f_2 axis, 90
f2coef parameter, 133
FAD technique, 181
fbc macro, 55
FDM (Filter Diagonalization Method), 68–71
fid file extension, 276, 282, 287, 288, 308
fiddc3d parameter, 131
fiddle command, 210, 211
Fiddle program, 210
fiddle program, 55
fiddle2D command, 212
fiddle2d command, 212
fiddle2Dd command, 212
fiddle2dd command, 212
fiddled command, 212
fiddleu command, 212
FIDs
    2D color intensity map, 113
    move between experiments, 32
    processing after each FID, 306
    varying one or more parameters, 32
File button, 124
Filter Diagonalization Method (FDM), 68–71
filter part numbers, 186
filters for indirect detection, 173
filtration for indirect detection, 186
final simulated spectrum frequency limits, 205
Find, 291
Find Entry button, 291, 291
first domain phasing, 98
first evolution time, 131
first point distortion, 98
fitspec command, 208, 208
fitspec.indata file, 207, 208
fitspec.inpar file, 207, 208
fitspec.outpar file, 207, 209, 209
fixed Gaussian fraction, 209
fla parameter, 240
flammable gases warning, 26
flow control meter, 260
flow rate caution, 436
flow rate parameter
    calibration, VAST, 351
flow rate setup
    faster runs, limited by the solution with the viscosity, 353
    optimized for maximum through put of samples with constant viscosity, 353
    slow runs, 352
fn parameter, 106
fn1 parameter, 106
fn2 parameter, 106
foldcc command, 114
folding about the diagonal, 114
folding along J=0 axis, 114
foldj command, 114
foldt command, 105, 114
forwards linear prediction, 108
Fourier transformation
    programs, 103
fp command, 38, 38
fpmult parameter, 98, 99
fpmult1 parameter, 98
fpmult2 parameter, 98
fractions, 414
frequencies, correcting, 55
frequency referencing, 114
frequency shift, 106
frequency-dependent phase shifts, 133
ft command, 35, 97, 98, 100
ft1d command, 103
ft1da macro, 104
ft1dac and ft2dac commands, 166
ft2d command, 98, 100, 103, 104, 106
ft2da macro, 103, 104
ft3d command, 131, 133, 134
full 2D display, 119
Full button, 117, 119, 124, 217
full command, 109
full screen with room for traces, 109
full width at half-height (FWHH), 121
fullt command, 109

G

g_array parameter, 43
g_max parameter, 43
g_min parameter, 43
g_steps parameter, 43
g2pul pulse sequence, 48
g2pulramp pulse sequence, 48
ga command, 261
gain parameter, 35, 303
GARP modulation, 173, 183
Gaussian apodization constant, 97
Gaussian function shift, 97
Gaussian functions, 95
Gaussian lines, 207
Gaussian lineshapes, 208
gaussian macro, 96
gcal parameter, 373
gcosy macro, 393
GCOSY pulse sequence, 393
GCU (gradient compensation unit), 372
generating weighting values, 96
getDshape statement, 247
getGshape statement, 247
getplane command, 132, 134
getRshape statement, 247
getsn macro, 302
getwet macro, 435
gf parameter, 95, 96, 97

gf1 parameter, 95, 96, 97
gf2 parameter, 97
gfs parameter, 97
gfs1 parameter, 97
gfs2 parameter, 97
ghmqc macro, 394
GHMQC pulse sequence, 394
ghmqcps macro, 394
GHMQCPS pulse sequence, 394
ghsqc macro, 395
GHSQC pulse sequence, 395
Gilson 819 valve
    Unit ID, 342
gilson command, 312, 317
glass wool plug, 263
global parameters, 297
globalauto parameter, 292
glue macro, 430
glue order, 324
gmapshim macro, 379, 383
gmapsys macro, 378, 378, 382, 383, 383, 388
gmapz macro, 304
gmqcosy macro, 396
GMQCOSY pulse sequence, 396
gnoesy macro, 397
GNOESY pulse sequence, 397
go command, 261
grad_cw_coef parameter, 44
grad_p_coef parameter, 44
grad_p1 parameter, 42
grad_p2 parameter, 42
grad_x parameter, 44
Gradient Autoshim on Z button, 379
Gradient Autoshimming Files menu, 383, 389, 392
gradient calibration constants, 44
gradient compensated stimulated echo w/ spin lock
    experiment, DOSY, 53
Gradient LC run, 413
Gradient Nucleus Parameter Setup menu, 389
gradient on flow data
    data processing, 414
gradient on flow experiment
    aborting, 414
gradient power levels, 44
gradient pulses, typical values for WET
    LC-NMR, 407
    VAST, 321
gradient runs, 410
gradient separation, 410
gradient shimming, 379
    pulse sequence, 380
Gradient Shimming menus
    Display, 389, 391
    Files, 389, 391
    Map, 389, 390
    Plot, 389, 392
    Setting, 389, 391
    Setup, 388, 390, 390
    System, 378, 388, 389
gradient stimulated echo element, 49
gradient systems, 371
gradient tables, 374
gradtype parameter, 371
grayctr parameter, 119
grayscale center control, 119
grayscale color set, 115
grayscale images threshold adjustment, 119
grayscale slope control, 120
graysl parameter, 119
grid lines, 112
grid macro, 112
gshimlib directory, 383
gtnnoesy macro, 398
GTNNOESY pulse sequence, 398
gtnroesy macro, 398
GTNROESY pulse sequence, 398
gxmax parameter, 375
gymax parameter, 375
gzmax parameter, 375
gzsize parameter, 388
gzwin parameter, 381

H

h1 macro, 302
h1p macro, 302
Hadamard transform, 240
halt command, 297, 302
hcchtocsy macro, 166
HCCHTOCSY pulse sequence, 166
Hd All button, 127
Hd Box button, 127
Hd Lbl button, 127
Hd Pk button, 126
HdNum button, 126
header parameter, 238
heat-exchanger coil in a water bath, 264
heating cycling, 264
helium contact with body, 26
helium gas flowmeters caution, 28
het2dj macro, 145
HET2DJ pulse sequence, 145
hetcor macro, 147
HETCOR pulse sequence, 147
hetcorps macro, 149
HETCORPS pulse sequence, 149
Heteronuclear 2D-J, 145
heteronuclear 2D-J data folding, 114
heteronuclear 2D-J experiment, 92, 103, 104, 105
Heteronuclear Chemical Shift Correlation, 147
heteronuclear chemical shift correlation, 94
Heteronuclear Chemical-Shift Correlation, Absolute
    Value and Phase Sensitive, 149
Heteronuclear Multiple-Bond Coherence, 174, 180
Heteronuclear Multiple-Quantum Coherence, 171,
    174
Heteronuclear Multiple-Quantum Coherence in
    Reverse Configuration, 176
heteronuclear Overbodenhousen experiment using
    REVINEPT, 199
heteronuclear shift correlation experiment, 100
heteronuclear spin-echo difference experiment, 177
high-power amplifiers cautions, 28
high-power decoupling and VT operation, 263
high-sensitivity protons, 171
high-temperature VT calibration, 264
HMBC pulse sequence, 174, 176, 180
HMQC

experiments, 171, 394
parameters, 184
phasing, 109
pulse sequence, 174, 177, 184, 194
hmqc macro, 174, 181, 188
HMQCR
parameters, 184
pulse sequence, 174, 176, 185
hmqcr macro, 174, 176, 181, 188
HMQC-TOCSY 3D sequence, 168
hmqctocsy macro, 168, 168
HMQCTOCSY pulse sequence, 168
HOHAHA pulse sequence, 164
HOM2DJ experiment, 151
hom2dj macro, 150
HOM2DJ pulse sequence, 150
homodec statement, 253
homonuclear 2D-J experiment, 94
homonuclear correlation phasing, 109
Homonuclear Hartmann-Hahn experiment, 164
Homonuclear J-resolved 2D, 150
homospoil gradient shimming, 385
homospoil gradient type, 375
horizontal axis, 111
HPLC detector, 415
HPLC system, 401
Hproj (max) button, 117
Hproj (sum) button, 117
hregions macro, 302
hsqc macro, 199
HSQC-TOCSY 3D sequence, 169
hsqctoxySE macro, 169
hypercomplex method, 92, 92
hyper-hypercomplex mode, 132

I

ice bath to cool VT gas, 264
il parameter, 35
image macro, 112
imageprint macro, 112
implicitly arrayed parameter, 92
Improving Results, suggestions, 387
INADEQUATE data folding, 114
INADEQUATE pulse sequence, 152
inadqt macro, 152
Incredible Natural Abundance Double-Quantum Transfer Experiment, 152
index of experimental frequency of a transition, 205
index2 parameter, 132
indirect detection experiments, 171
indirect detection probe, 172
Indirect•nmr™ probe, 172
indirectly detected axis, 111
inept macro, 82
INEPT pulse sequence, 79, 82
Info button, 126, 128
info_n file, 44
initialize VAST, 315
initialize_iterate macro, 205
injector valve, 342
ins parameter, 121
Insensitive Nuclei Enhanced by Polarization Transfer, 82
insert peak at cursor position, 122, 125
inssref parameter, 121
instrumental errors, 210
integral, 430
integral resets, 100
intensity of calculated transitions, 205
intensity threshold for simulated spectrum, 205
interactive
2D peak picking, 120
add or delete peak bounds, 128
display 2D traces and projections, 115
parameter arraying, 34
spectra add/subtract, 216
weighting parameters, 96
Interactive 2D Color Map Display Main Menu, 117
Interactive 2D Display Projection Menu, 117
Interactive Mode button, 216
interferogram left-shift, 106
interferogram phase rotation, 106
interferograms, 89
interleaved acquisitions, 35
intmod parameter, 36, 100
inversion pulse creation, 241
inversion-recovery T_1 experiment, 37
is parameter, 121
isocratic data processing, 430
isocratic LC run data processing, 413
isocratic LC runs, 411
isocratic runs, 410
iterate parameter, 205
iterative mode of spin simulation, 205
iterative spin simulation, 201

J

jexp command, 302
jumpret macro, 83

K

ketone solvents in coolant bucket, 261
killft3d macro, 132
kind macro, 40
kinds macro, 40
kinetics data analysis, 218
kinetics studies, 39
kini macro, 40
kinis macro, 40

L

L.O. frequency, 172
L.O. SELECT switch, 173
Label button, 125, 128
label for a peak, 122
LAME program, 201
LAOCOON program, 201
last lock solvent, 309
lastlk file, 309
lb parameter, 95, 96, 97
lb1 parameter, 95, 96, 97
lb2 parameter, 97
LC column, 436

lc1d macro, 455
LC1D pulse sequence, 455
lc1d sequence pane, 404, 405
LC-NMR
  1D pulse sequence, 455
  Analyte Collector, 427
  data processing, 429–431
  delay time calibration, 431
  delay time calibrations, 431
  in VNMR, 401
  multiple frequency suppression options, 406
  new solvent concentration, 435
  ProStar 330 transfer tube, 432
  solvent mixture equilibration, 408
  solvent suppression problems, 408
  solvent suppression setup, 404
  solvent suppression, retrieving parameters for, 404
  standard compounds, 435
  system, 401
  WET shapes, 409
LCNMR/STARS tab, VAST, 316
lcqueue file, 415
least squares curve-fitting method, 218
left command, 109
left shift interferogram, 106
lfs (low-frequency suppression), 106
line broadening factor, 97
line number table, 204
line positions, 206
line shapes, correcting, 55
linear amplifier systems, 173
linear prediction, 107
  processing control, 105
linewidth for deconvolution calculations, 208
linewidth of simulated spectrum, 205
Liquid Handler, 355
liquid handler
  air connections, diagram, 343
  connecting signal and power cables, 342
  signal cable connections diagram, 344
liquid handler arm
  XYZ coordinates, adjusting, 361
Liquid Handler window, 312, 317
liquid handler window, typical default values, 313
liquid nitrogen, 263, 265
list button, 206
list of line frequencies, 206
literature references, 94
ll2d command, 118, 120
ll2d directory, 120, 129
ll2d file, 126
ll2dmode parameter, 122, 125, 129
llfrq parameter, 206
loc parameter, 267
loc, as used with VAST, 314
local oscillator frequency, 172
local oscillator signal, 183
lock interlock, 265
lock pin, 270, 270
locked mode, 270
lockpower parameter, 303
locQ file, 296
log file, 308
long-range HMQC pulse sequence, 174
loop collection runs, 414
loop collector valve, 452
Lorentzian lines, 207
Lorentzian lineshapes, 208
low-temperature VT calibration, 264
lp parameter, 98, 99
lp1 parameter, 98, 108, 109
lp2 parameter, 98, 129
lsfid parameter, 214
lsfid1 parameter, 106
lsfid2 parameter, 129
lsfrq parameter, 106, 132
lsfrq1 parameter, 106, 132
lsfrq2 parameter, 106, 132

M

macromolecules, 180
macros
  acquisition, 301
  customizing macro operation, 301
  glue, 430
magnet quench warning, 26
magnetic equivalence factoring, 201
magnetic media caution, 27
magnitude-mode 2D experiment, 104
magnitude-mode transform, 104
Main Control, gilson window, 355, 359
make3dcoef macro, 133
manual sample change
  VAST, 314
mapping the shims, 377
Marion and Wuthrich, 92
Mark button, 125, 128, 209
mark button, 206
mark command, 118, 207
mark1d.out file, 206, 207, 209
match experimental and calculated lines, 205
maximum intensity, 111
maxincr parameter, 237
measured line frequencies, 205
metal objects warning, 25
methanol for VT calibration, 264
method building with STAR 5.3, 438
Microflow probe, 401
  connecting air supply from Valco valve, 342
microflow probe and transfer tube volumes, 345
microflow probe cell, 348
min button, 217
mix parameter, 158
mixing patterns creation, 230
modifying the instrument, 26
modulated decoupling, 183
move
  last FID and parameters, 32
  parameters between experiments, 32
mp command, 32
mqcosy macro, 153
MQCOSY pulse sequence, 153
multiexperiment mode, 296
multi-FID add/subtract experiment, 214
multiple arrays of parameters, 33
multiple frequency suppression options

LC-NMR, 406
VAST, 319
Multiple-Quantum Filtered COSY, 153
multiply first point, 98
multiply selective adiabatic inversion pulse creation, 242
multiply selective pulse creation, 240

N

name parameter, 237
NeedleRinseRate, 359
NeedleRinseVolume, 359
negative intensities, setting, 114
nested supercycle, 241
nextpl macro, 132
ni dimension, 95
ni parameter, 89, 90, 96, 129, 130, 132
ni2 parameter, 96, 106, 129, 130, 132
ni3 parameter, 134
niter parameter, 205
nitrogen contact with body, 26
nitrogen gas caution, 260
nitrogen gas flowmeters caution, 28
nitrogen gas for VT use, 260
nlivast, 338
nlivast2, 338
nlivast3, 339
nll command, 206
nm2d command, 112
NMR response, 430
NMRgram, 430
NOE difference experiment, 84, 84
NOEDIF experiment, 84
noedif macro, 84
NOESY
data folding, 114
data symmetrization, 114
phasing, 109
pulse sequence, 155
noesy macro, 155
noise reject parameter
LC-NMR, 408
VAST, 321
noise suppression, 119
non-whitewashed stacked spectra, 113
notational conventions, 29
npoint parameter, 38
nt parameter, 35
nt_array parameter, 43
nt_first parameter, 43
N-type data, 400
N-type experiment, 104
N-type peak selection, 103
N-type peaks, 104
Nuclear Overhauser Effect Spectroscopy, 155
Nuclear Overhauser Enhancement difference experiment, 84
null string, 307
number of increments, 89
number of iterations, 205
NumRinses, 357

O

observe channel, 172
observe pulse and power
LC-NMR and solvent suppression, 404, 406
VAST and solvent suppression, 319
off-resonance SEDUCE-1 decoupling, 241
ofs parameters, 239
oneshot DOSY experiment, 54
on-flow experiments
column overload, 411
on-flow LC experiments, 410–414
acquisition time, 411
gradient, 410, 413
gradient, aborting, 414
isocratic, 410, 411
isocratic, aborting, 412
large injection, 411
number of transients/spectra, 411
sensitivity, 411
signal strength versus resolution, 411
width of the peaks, 411
on-flow LC-NMR, 401
operating the amplifier for PFG, 373
optical sensor, 272
opx macro, 232, 242
order of 2D experiments, 93
origin for phasing, 134
output transistor failure on VT controller, 264
over-temperature circuit, 265
oxidation of heater and thermocouple, 263

P

p31p macro, 302
pa command, 110
pa1 command, 109, 110
pacemaker warning, 25
pacosy command, 115
pad parameter, 39, 261
pad parameter, 299
padept command, 82
paired data, 218
Pandora's Box, *See* Pbox
par2d macro, 90
par3d macro, 106
par4d macro, 134
parameters
array format, 32
array order and precedence, 34
Dbppste, 53
Dbppsteinept, 55
Dgcstecosy, 59
Dgcstehmqc, 60
DgcsteSL, 53
FDM, 69
interactive arraying, 34
iterated, 205
jointly arrayed, 34
move between experiments, 32
move saved display, 32
multiple arrays, 33
oneshot DOSY, 54
parim macro, 120

parlib directory, 73
parll2d macro, 123
parlp macro, 105, 108
path3d parameter, 131
Pbox, 230
  macros reference, 242
  menus, 231
  miscellaneous PSG statements, 253
  parameter list, 234, 237
  PSG statements, 243, 247
  pulse calibration numbers, 231
  wave definition strings, 234
Pbox command (UNIX), 233, 258
Pbox.inp file, 232, 233, 233
pbox_bw macro, 243
pbox_bws macro, 243
pbox_dec2off statement, 251
pbox_dec2on statement, 250
pbox_dec2pulse statement, 248
pbox_dec3off statement, 251
pbox_dec3on statement, 251
pbox_dec3pulse statement, 249
pbox_decoff statement, 250
pbox_decon statement, 250
pbox_decpulse statement, 248
pbox_dmf macro, 232, 243
pbox_dres macro, 243
pbox_drex macro, 232
pbox_files macro, 243
pbox_grad function, 247
pbox_grad statement, 252
pbox_name, 243
Pbox_psg.h include file, 244
pbox_pulse function, 244
pbox_pulse statement, 248
pbox_pw macro, 232, 242
pbox_pwr macro, 232, 243
pbox_pwrf macro, 243
pbox_rst macro, 232, 243
pbox_sim3pulse statement, 249
pbox_sim4pulse statement, 249
pbox_simpulse statement, 249
pbox_xgrad statement, 252
pbox_xmtroff statement, 250
pbox_xmtron statement, 250
pbox_ygrad statement, 253
pbox_zgrad statement, 253
pboxget macro, 242
pboxpar macro, 232, 242
pcon command, 112
pcss.outpar storage file, 224
Peak button, 117, 123, 124, 127
peak comment, 126
peak file information, 126
peak files, 121
peak height, 38, 430
peak intensities, 38
peak label, 125, 128
peak volume, 124
peak(s)
  selecting solvent for suppression, VAST, 316
peak2d command, 111
peaks(s)
  selecting for solvent suppression using sequence pane, LC-NMR, 406
  selecting in sample entry window for suppression, LC-NMR, 419
peaks.bin file, 120, 126, 126
peaks_f#f#_#.bin file, 121
peek tubing, I.D. and volume calculations, 341
perform spin simulation, 205
Performa I PFG module, 371
  amplifier lights, 373
  parameter settings, 372
  shimming, 374
Performa II PFG module, 40, 371
  amplifier lights, 373
  parameter settings, 372
  reset button, 373
  shimming, 374
Performa III PFG module, 371
  amplifier lights, 373
Performa XYZ PFG module, 371
  amplifier lights, 373
  amplifier-produced quiescent current, 372
  parameter settings, 372
  shimming, 374
pexpl command, 38, 47, 220
pexpladd macro, 47
PFG Absolute Value COSY, 393
PFG Absolute Value MQF COSY experiment, 396
PFG Absolute-Value ROESY experiment, 398
PFG HMQC experiment, 394
PFG HMQC, Phase Sensitive, 394
PFG HSQC experiment, 395
PFG NOESY experiment, 397
PFG Selective Excitement, 400
PFG systems
  experiments, 393
  gradient subsystem, 371
  user interface, 372
PFG TNNOESY experiment, 398
pfg_pulse statement, 253
pfgon parameter, 372, 372, 374, 374
pge macro, 43
pge parameter, 48
pge parameter set, 42
PGE pulse sequence, 41, 42
pge_calib macro, 44
pge_data macro, 44
pge_output macro, 47
pge_process macro, 44
pge_process macro, 44
pge_results macro, 45, 47
pge_setup macro, 43, 44
pgeramp pulse sequence, 48
ph command, 97
ph parameter, 239
ph1 command, 97, 109
phase angle mode, 109
Phase button, 108
phase correction, 108
phase cycling, 105
phase parameter, 92, 132, 132, 133, 181
phase parameters, 98
phase rotate interferogram, 106
phase shift the receiver, 105
phase2 parameter, 129, 132, 133

phase3 parameter, 134
phased color set, 115
phased data, 91
phases, correcting, 55
phase-sensitive 2D NMR, 91
phase-sensitive COSY pulse sequence, 141
phase-sensitive HETCOR, 149
phase-sensitive mode, 109
phase-twist, 104
phasing corrections, 109
phfid parameter, 214
phfid1 parameter, 106
phfid2 parameter, 129
phi parameter, 229
pi3ssbsq macro, 96
pi4ssbsq macro, 96
pl command, 36, 113
pl2d command, 113
plane parameter, 134
plate_glue, 327, 339
pldept macro, 82
plfit macro, 209
plgrid macro, 112
pll2d command, 120, 129
plot
    2D traces and projections, 115
    connectivities, 115
    deconvolution analysis, 210
    exponential curves, 38
    grid lines, 112
    image, 112
    limits, 109
    non-whitewashed stacked spectra, 113
    polynomial curves, 38
    regression analysis, 220
    results of 2D peak picking, 120
    series of 3D planes, 132
    spectra, 36
    spectra with whitewashing, 113
Plot button, 117, 117, 210
plplanes macro, 132
Plunger, 361
plvast, 326, 339
plvast2d, 326, 339
plvastget, 326, 326
pmode parameter, 97, 109, 133, 134
point-by-point intensity of the spectrum, 207
polarization transfer experiment, 81
poly0 macro, 220
polynomial curves analysis, 38
polynomial fitting, 100
power mode, 109
power switch (VT unit), 260
pph macro, 243
pprofile macro, 243
pre_sat statement, 254
preacquisition delay, 39, 261
predicting variables, 218
preparing VAST for use, 312
presat macro, 87
presat statement, 253
presaturation mechanism, 180
prevpl macro, 132
Prime pump, 361
probe
    directory, 73, 137
    fast rate, 351
    guide holes, 272
    impedance mismatch, 48
    pulsed Z-gradient, 40
    slow rate, 351
    slow vol, 351
    temperature, 259
    tuning in reverse mode, 186
probe volume, 313
    calibration, VAST, 345
    control data set, obtaining using a syringe, 346
    finding optimum value, 349
    initial estimates, 348
    VAST, 345
    verifying using liquid handler, 347
ProbeFastRate, 358
ProbeSlowRate, 358
ProbeSlowVol, 358
ProbeVolume, 357
proc parameter, 105
proc1 parameter, 93, 105, 108
proc2 parameter, 107, 108
processing
    2D DOSY experiments, 55
    3D-DOSY experiments, 61
processing stored 2D data, 90
processing type, 105
processing, displaying, and plotting
    from glued data sets, 325–326
    isocratic LC-NMR data, 430
    macros for VAST data, 337
    VAST data sets, 323–325
procplot macro, 301
profile-type experiments, 376
Proj button, 117, 117, 119
proj command, 113
project data onto axis, 113
Projection Menu, 119
ProStar 330, delay times, 432
prosthetic parts warning, 25
protective relay on VT controller, 265
proton 2D-J spectra, projecting, 114
pseudo 2D data set
    creating from a VAST data set, 323
    creating from an isocratic LC-NMR experiment, 430
pseudo macros, 96
pseudo-2D, 340
pseudo-echo weighting, 95
psglib directory, 73, 138
psgQ file, 294, 296, 308
pshape macro, 243
ptspec3d parameter, 131
P-type data, 400
P-type experiment, 104
ptype parameter, 239
P-type peak selection, 103
pulse creation routine, 230
pulse sequence variations, 48
pulse sequences
    3D DOSY, 58
    Dgcstecosy, 58

Dgcstehmqc, 58
DOSY, 48, 51
pulse template file, 225
pulse width calibration, 37
Pulsed Field Gradient experiments, 393
pulsed field gradient shape creation, 230
pulsed gradient experiments, 40
pulsed Z-gradient probe, 40
pulsetool command, 225
Pulsetool program, 225
pure 2D absorptive lineshapes, 97
pure absorption spectra, 104
Push Volume, 350
PushVolume, 357
putwave macro, 242
pw parameter, 239
pwr1 command, 109
pwxcal macro, 190
Pxfid command (UNIX), 258
pxshape macro, 232, 242
Pxsim command (UNIX), 258
Pxspy command (UNIX), 258

Q

queueing experiments, 267

R

r macro, 108
Rack Def., gilson window, 355, 359
Rack Definition, 312
radio-frequency emission regulations, 28
ramp length, 48
rate of VT gas flow, 263
react macro, 302
Read button, 126
Read Text button, 126
reading
binary peak file, 126
text peak file, 126
real FT processing, 105
real-time 2D, 94
Redraw button, 117, 119
ref_pw90, 317
LC-NMR, 404
VAST, 317
ref_pw90 parameter, 239
ref_pwr, 317
LC-NMR, 404
VAST, 317
ref_pwr and ref_pw90 calibration data, 231
ref_pwr parameter, 239
reference deconvolution, 55, 210
reference literature, DOSY-related, 67
referencing, clearing in 2D spectra, 114
refocusing pulses creation, 241
refofs parameter, 238
refpos1 parameter, 114
refrigerating device, 264
region-selective 3D processing, 131
regionx_results file, 47
regression, 218
regression analysis, 218
regression.inp file, 218, 219, 221, 222, 223
relaxation times measurement, 38
relaxation-sensitive simulation, 241
RELAY-COSY pulse sequence, 138
relayh macro, 138, 471
relief valves warning, 27
removable quench tubes warning, 27
removing systematic errors, 55
reps parameter, 238
Reset arm, 361
Reset button, 125
reset parameters, 134
resetf3 macro, 134
resolution enhancement, 95, 97
restore spin system to before last iterative run, 206
resume command, 302
retaining disk, 270
Return Home, 361
Return key, 30
reverse configuration, 172, 174
review papers, DOSY, 68
REVINEPT, 199
rf generation for transmitter and receiver, 172
rf signal routing for indirect detection, 173
rf system of spectrometer, 172
rfl parameter, 114
rfl2 parameter, 129
rfp parameter, 114
rfp2 parameter, 129
Rheodyne Injector Valve
bypassing, 342
connecting transfer tubing, 341
right command, 109
rinput macro, 219
RinseDeltaVol, 358
RinseExtraVol, 359
roesy macro, 158
ROESY phasing, 109
ROESY pulse sequence, 158
room temperature stability, 264
rotate command, 114
rotate homonuclear 2D-J data, 114
rotated homonuclear 2D-J data folding, 114
Rotating Frame Overhauser Experiment, 158
rp parameter, 98
rp1 parameter, 98, 108, 108
rp2 parameter, 98, 129
RS-232C serial link, 279
rt command, 36
rubber stopper, 272
run mode, 270

S

s macro, 32
s2pul macro, 87, 183
S2PUL pulse sequence, 87, 174, 182
s2pulr macro, 88, 174, 194
S2PULR pulse sequence, 88, 174, 185
safety circuits on VT controller, 264
safety precautions, 25, 27
safety sensor for VT controller, 264
sample
adjusting liquid height, 280

change cycle time, 279
location, 267
positioning in spinner, 280
positions, 279
protocols, 363
removal errors, 283
spinning, 263
temperature, 262
sample changers, 267
sample preparation, 280
Sample Def,
gilson window, 356
SAMPLE Def.
field definitions, 356
gilson window, 355
sample entry form window
LC-NMR, 419
VAST, 315
sample tray, 279
sample location, 295
sample locations, 294
Sample Volume, 348
sample volume, calibrate, 350
sample zero, 279
SampleDepth, 359
SampleExtraVol, 358
SampleHeight, 359
sampleinfo file, 296, 297, 308
SampleKeepFlag, 357
SampleVolume, 357
SampleWellRate, 359
satellite signals, 212
save button, 217
saved display parameters, 32
saveglobal parameter, 297, 300
sb parameter, 96, 97
sb1 parameter, 96, 97
sb2 parameter, 97
sbs parameter, 96, 97
sbs1 parameter, 96, 97
sbs2 parameter, 97
sc parameter, 36, 109
sc2 parameter, 36, 109
scalelimits macro, 38, 220
Scout Scan, 417
LC-NMR, 413
VAST, 321
Scout Scan data processing, 430
Scout Scan solvent suppression, LC-NMR, 411
sdp macro, 58
sealed samples at elevated temperatures, 262
sealed samples caution, 265
second cursor pair, 118
second evolution time, 131
SEDUCE-1 decoupling, 241
Select button, 217
selecting composite observe pulse
LC_NMR, 407
VAST, 320
selecting solvents for suppression
LC-NMR, 404
VAST, 318
selective excitation, 230, 400
selective Fourier transformation, 104
selex macro, 232, 242
selexcit macro, 400
sensitivity
analyte concentration, 410
band broadening, 410
gradient separation, 410
maximizing, LC-NMR, 410
minimizing tubing volume, 410
on-flow experiments, 410, 411
sample injection, 410
stopped-flow experiments, 410, 416
sensitivity enhancement, 97
Set Params button, 205, 205
set3dproc command, 133
setgauss macro, 209
setgcoil macro, 375
sethw command, 260, 295
setlimit statement, 254
setlk macro, 301, 302, 302, 303
setref macro, 114
setref1 macro, 114
setref2 macro, 114
setscout macro, 435
setsw1 macro, 114
setting up
2D-DOSY experiments, 51
3D_DOSY experiments, 58
setvalue command, 215
setwave macro, 232, 242
setwet macro, 435
sfrq parameter, 238
Sh All button, 127
Sh Box button, 126
Sh Lbl button, 127
sh parameter, 239
Sh Pk button, 126
shape information, 242
shape macro, 243
shape statement, 247
shaped gradients creation, 241
shaped pulses, 225
shaped rf pulses creation, 230
shapedgradient macro, 247, 247
shapelib directory, 233
shift frequency of spectrum, 132
shim field maps, 380
shimmaps
displaying, 381
distributing to users, 383
loading, 382
shimming PFG systems, 374
ShNum button, 126
showfit macro, 209
showing diffusion projection, 58
sidechain assignments in fully ^{13}C-enriched
biopolymer, 166
sign of frequencies, 105
sign reversals along f_1, 105
signal-to-noise estimate, 302
simple excitation pulse, 240
simulated spectrum frequency limits, 205
sine macro, 96
sine window function, 96
sinebell

macro, 96
shift, 97
time period, 97
weighting, 95
sinebell-squared weighting, 95
sinesq macro, 96
sine-squared window function, 96
single-broadband systems, 172
recabling for indirect detection, 185
single-user sample changer operation, 287
skyline projection, 113
slfreq parameter, 205, 206
slice width value, 416
SLP, 167
slpwr parameter, 158
slw parameter, 205, 208, 209, 209
smaxf parameter, 205, 206
sminf parameter, 205, 206
SMS sample changer, 267
sn parameter, 302
solids high-power amplifiers caution, 28
solvent information, LC-NMR, 413
solvent mixture equilibration
LC-NMR, 408
VAST, 322
solvent subtraction 2D transform, 106
solvent suppression, 404
LC-NMR acquisition and processing options, 407
LC-NMR post acquisition signal suppression, 407
retrieving parameters for, LC-NMR, 404
retrieving parameters for, VAST, 317
Scout Scan, LC-NMR, 411
VAST, 317
VAST acquisition and processing options, 320
VAST post acquisition signal suppression, 320
wet width, LC-NMR, 407
wet width, VAST, 320
solvent suppression background information
LC-NMR, 409
VAST, 322
solvent suppression problems
LC-NMR, 408
VAST, 321
sp parameter, 111, 119
sp wp button, 217
sp1 parameter, 111, 119
sp2 parameter, 129
spadd command, 215
specdc3d parameter, 134
spectral
display, 35
drift correction, 134
plot, 36
spectral width, setting the, 114
spectroscopy, 2-D DOSY, 51
spectrum
deconvolution, 208
move cursor to center, 114
spin lock, 158
spin simulation, 201
spin simulation algorithms, 201
Spin Simulation Line Assignment Menu, 206
spin-echo difference experiment, 177
spini.indata file, 206
spini.inpar file, 206
spini.la file, 206, 206
spini.outpar file, 206
spini.savela file, 206
spinll macro, 206, 206
spinner air supply, 263
spins command, 205, 205, 206
spins.inpar file, 207
spins.list file, 205, 206
spins.outdata file, 207
spins.stat file, 207
spline fitting, 100
spsm command, 204
spsub command, 215
sqcosine macro, 96
sqsinebell macro, 96
ssfilter and solvent suppression
LC-NMR, 409
VAST, 322
ssfilter parameter, 106
ssorder parameter, 106
st parameter, 239
Standard Two-Pulse in Reverse Configuration, 88
Standard Two-Pulse Sequence, 87
Standard Two-pulse with Decoupler as Transmitter, 78
STAR 5.3 software
building a method, 438
loading, 436
STAR Chromatography
setting up an automation run, 441
STAR chromatography program, 435
STAR Chromatography software, 401
start of chart, 109
starting point for a deconvolution, 209
States, Haberkorn, and Ruben, 92, 104
status command, 294, 297
stdpar file, 301
stdpar parameters, 297
steps parameter, 237
stepsize parameter, 238
sth parameter, 205, 205, 206
stim parameter, 42
stimulated echo experiment, 42
stop-flow experiments LC-NMR, 401, 414–420
how stop-flow LC-NMR works, 415
maximizing analyte concentration, 416
sensitivity, 416
slice width, 416
threshold value, 416
stop-flow time slice experiments, see
time slice stop-flow LC-NMR
store line assignments, 206
su command, 261, 263
sub button, 217
sub command, 214
subexperiments, 32
subtract FID from add/subtract experiment, 214
sucyc parameter, 238
summing projection, 113
Sun-based VXR-5000 data system, 174
supercycling, 241

superhypercomplex data acquisition, 129
supply air pressure variations, 272
suppression of
    13C satellites, LC-NMR, 406
    13C satellites, VAST, 319
svf command, 36
svs parameter, 205
sw parameter, 239
sw1 parameter, 89, 129, 130
sw2 parameter, 106, 129, 130, 132
sw3 parameter, 134
switchable probes, 172
switchable relays, 173
symbol, 34
symmetrize data, 114
Syringe Volume, 361
sysgcoil parameter, 375
System Configuration window, 447
systematic errors, removing, 55

T

T_1 and T_2 NMR data analysis, 218
t1 command, 38
t_1 domain, 95
T_1 experiment, 38
T_1 exponential time, 37
T1 parameter, 238
t1s command, 38
t2 command, 38
T_2 experiment, 38
T_2 measurement, 76
T2 parameter, 238
t_2 time, 90
t2s command, 38
TANGO pulse, 394
Tcl-Tk acquisition panel, Dgcstecosy pulse sequence, 59
Tcl-Tk acquisition panel, Doneshot pulse sequence, 52
Tcl-Tk process panel, 2D_DOSY pulse sequences, 57
Tcl-Tk process2 panel, 3D_DOSY pulse sequences, 62
temp command, 262
temp parameter, 32, 259, 261, 261
tempcal command, 264
temperature calibration curve, 264
temperature control, 261
temperature control window, 263
temperature interlock parameter, 265
temperature limit sensor, 265
temperature readout, 261
temperature regulation, 265
testct macro, 302
testsn macro, 302
text peak file, 121, 126
th parameter, 112, 112, 204
th2d parameter, 122, 124, 128
theoretical spectrum for spin 1/2 nuclei, 201
thermocouple, 259, 264
theta parameter, 229
third indirectly detected dimension, 135
threshold for integrating peaks, 122
threshold value, LC-NMR, 416
time delay calibration, 431–435
time periods in the 2D experiment, 90
Time Program window, 450
Time Proportional Phase Incrementation, 92
time slice program, 421
time slice stop-flow LC-NMR, 421–425
    fully automatic data acquisition, 421
    manual data acquisition, 425
    semiautomatic data acquisition, 422
    time slice program, creating in Cascade, 421
time-domain frequency shifting, 132
tin parameter, 265, 298
TMS satellite signals, 212
tncosyps macro, 160
tndqcosy macro, 160
tnmqcosy macro, 161
tnnoesy macro, 161
tnroesy macro, 162
tntocsy macro, 163
tocsy macro, 164
TOCSY phasing, 109
TOCSY pulse sequence, 164
tools, DOSY, 49
Total Correlation Spectroscopy, 164
TPPI experiment, 92
TPPI method, 92, 93
TPPI phase cycling, 133
Trace button, 117, 119
trace parameter, 96, 109, 111, 112, 113, 134
tramp parameter, 48
transfer tube
    calculating the volume, VAST, 341
transform non-arrayed 2D data, 106
transition amplitude of calculated transitions, 205
Transverse ROESY, 166
trev parameter, 240
Trial WET
    LC-NMR, 404, 407, 408
    VAST, 317, 321, 322
triangularize 2D spectra, 114
Triple•nmr™ probe, 172
triple-resonance probe, 172
trise parameter, 375
troesy macro, 166
TROESY pulse sequence, 166
true contour plot, 115
true contour plot display, 112
truncation wiggles, 95
two-component analysis, 47
type parameter, 237
typical flow rates for common solvents, 353

U

undospins macro, 206, 206
UNIT ID of Gilson 819 valve, 342
Unmark button, 125, 128
upfield peak shift, 106
upper barrel warning, 26
Use Mark button, 207
usemark macro, 207, 208

V

Valco valve
  connecting air tubing, 342
variable temperature, *See* VT
Varian LC-NMR system, 401
VAST
  air connections, 343
  autosampler, 267, 355
  data analysis, 337
  files that control operation, 363
  Hardware installation and configuration, 341–362
  interface to VNMR, 355
  microtiter plate, 337
  multiple frequency suppression options, 319
  Operation and data processing, 311–341
  sample entry form window, 315
  sequence pane, 317
  set up NMR experiments, 314
  shut down, 316
  signal cable connections, 344
  solvent mixture equilibration, 322
  solvent suppression problems, 321
  solvent suppression setup, 317
  solvent suppression, retrieving parameters for, 317
  transfer tube volume, 341
  WET shapes, 322
  writing protocols, 363
VAST data sets, examples of plots
  96 1D spectra plotted using plvast, 328
  using a custom display order, 328
vastget, 326, 340
vastglue, 324, 340
vastglue2, 325, 340
versatile automatic sample transport, 311
vertical scale adjustment, 119
vertical temperature gradient, 263
vloc
  as used with VAST, 314
Volume button, 124, 128
vortex plug, 263
Vproj (max) button, 117
Vproj (sum), 117
vs parameter, 112
vs2d parameter, 112, 121
VT accessory, 259
VT Acqmeter display, 260
VT controller, 262
VT Controller label, 260
VT controller power, 260
VT controller safety circuits, 264
VT cutoff, 260
VT experiment warning, 26
VT FAILURE message, 265
VT gas flow rate, 263
VT indicator light, 261, 262
VT nitrogen gas flow, 261
VT operation, 259
VT operation with a sample changer, 298
VT regulation light, 265, 265
vtc parameter, 259, 261
vtwait parameter, 263, 265
VXR-4000/Gemini data system, 174

W

WALTZ decoupling, 173
WALTZ-4 modulation, 183
warnings defined, 25
water eliminated through transverse gradients 1D, 457
water in VT exchanger, 262
water suppression, 75, 87
wave definition string, 233
wave string variables, 239
waveform generation, 230
wavelib directory, 233
wbs parameter, 306
wc parameter, 36, 109
wc2 parameter, 36, 109
weighting values generation, 95
werr parameter, 297, 306
WET
  double relay-COSY, 471
  double-quantum filtered COSY, 458
  LC-NMR, 404
  Nuclear Overhauser Effect spectroscopy, 466
  PFG absolute-value COSY, 460
  PFG Absolute-Value MQF COSY, 465
  PFG HMQC, 462
  PFG HSQC, 463
  pulse width X channel calibration, 469
  relay-COSY, 471
  TOCSY with water suppression, 474
  VAST, 318
WET experiments
  solvent suppression, LC-NMR, 409
  solvent suppression, VAST, 322
WET shapes
  LC-NMR, 409
  VAST, 322
Wet Width
  LC-NMR, 407
  VAST, 320
wet1d macro, 457
WET1D pulse sequence, 457
wetcosy macro, 471
wetdqcosy macro, 458
WETDQCOSY pulse sequence, 458
wetgcosy macro, 460
WETGCOSY pulse sequence, 460
WETGHMQCPS pulse sequence, 462
wetghsqc macro, 463
WETGHSQC pulse sequence, 463
wetgmqcosy macro, 465
WETGMQCOSY pulse sequence, 465
wethmqcps macro, 462
wetnoesy macro, 466
WETNOESY pulse sequence, 466
wetpwxcal macro, 469
WETPWXCAL pulse sequence, 469
WETRELAYH pulse sequence, 471
wettntocsy macro, 474
WETTNTOCSY pulse sequence, 474
wexp parameter, 299, 307
WFG (waveform generator), 371
wft command, 35, 97, 100
wft1d command, 103, 134
wft1da macro, 104

wft1dac and wft2dac commands, 166
wft2d command, 94, 100, 103, 104, 133, 134
wft2da macro, 104, 104, 106
wft2dac macro, 103
wftt3 macro, 131
whitewashed stacked plot, 115
whitewashing, 113
width of chart, 109
wnt parameter, 306
Workspace button, 32
Workspace Menu, 32
wp parameter, 111, 119, 218
wp1 parameter, 111, 119
wp2 parameter, 129
wrap parameter, 238, 240
Write Text button, 126
writing VAST protocols, 363
wshim parameter, 379
wti command, 96, 134
wtia command, 97

X

X Axis, Y Axis, Z Axis label in CONFIG, 371
X-approximation, 201
X-axis label, 223
xdiag parameter, 122
X-nucleus decoupling, 182
X-nucleus decoupling frequency, 172
XYZ coordinates of the liquid handler arm, 361
XYZ coordinates of the rinse station, 354

Y

Y-axis label, 223

Z

z0 parameter, 303
Z1 room temperature shim as a gradient, 385
Z1 shimming shift, 372
zeroneg command, 114
zero-order phase rotation, 106
zfs (zero-frequency suppression), 106